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STUDIES IN CROP PHYSIOLOGY

Effect of Nitrogen, Phosphorus and Potassium Deficiencies on Respiration Rate of Sugarcane Leaves

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Introduction

Recent investigations have shown that the rate of carbon dioxide evolved is correlated with the amount of nutrients supplied to plants.[1, 2, 3, 14, 18, 28] Of these, nitrogen is noted to increase respiration.[17, 19] High respiration in nitrogen-fed plants is generally noted when plants contain an initial carbohydrate reserve[7] and is associated with low electrical specific resistance.[17] Changes in chemical composition are also characteristically noted, in response to added nitrogen.[4, 5, 6, 9, 10, 11, 12, 16] In nitrogen starved plants on the other hand, low respiration[4] is suggested to depend upon two opposing tendencies, *viz.*, that of low nitrogen to depress respiration and of high carbohydrates to accelerate it.[31] Reduction in respiration is also related to low enzymic activity and low protein content in nitrogen-deficient plants.

Phosphorus increases respiration during early growth.[13, 25, 26] Its effect is subsequently reversed at later periods.[19] In seedlings, phosphorus increases respiration which is limited, not by a lack of available carbohydrates but by a lack of phosphorus.[8] Supplied alone or in combination with nitrogen, it depresses respiration of field crops.[17] Low respiration, phosphorus supply and high specific resistance appear related.[17] Deficiency of phosphorus results in high reducing sugars, high sucrose and high total carbohydrates.[11, 12, 15] In phosphorus-deficient barley low sugar is associated with low respiration, low protein synthesis, poor meristematic activity, low assimilation and high translocation.[5,23]

Potassium is known to regulate carbohydrates[21, 29] by activation of amylolytic activity.[30] Reduced potassium supply is associated with low carbohydrates[20] and decrease in soluble sugars and starch.[10, 11, 12] Its deficiency on the contrary, is associated with low sugar and high amino-nitrogen,[6,23] high starch, low assimilation and poor enzymic activity. It also causes super-normal respiration[4] and subnormal photosynthesis. In

general, nutrients producing plants with external symptoms of potassium deficiency, show supernormal respiration which is invariably related to protein content.[24]

It is thus obvious that respiration is markedly altered in response to presence or absence of nitrogen, phosphorus or potassium and not infrequently, the recorded variations are explained on the basis of observed changes in carbohydrate and nitrogen fractions of the tissues. How far deficiencies of these ingredients affect respiratory activity of sugarcane leaves is described in these pages.

Methods and Material

Comparative study of respiratory activity of successive leaves on the main shoot of five sugarcane varieties, *viz.*, Co 453, Co 421, POJ 2878, Co 205, and Rheora, was carried out towards the maturity stage. Seedlings of these varieties were raised in sand in glass-house under adequate soil moisture. Healthy germinated buds, 15 days old, were carefully planted in pots filled with sand (80 lb.), at the rate of one per pot. Soon after establishment of germinated sets, nutrient solutions were added; as many as four series of cultures namely, complete nutrition (C), nitrogen deficiency (— N), phosphorus deficiency (— P) and potassium deficiency (— K) were maintained. These received the following quantities of salts:

Quantity of nutrients in grams per pot*

No.	Salts	Complete nutrition	Nitrogen deficiency	Phosphorus deficiency	Potassium deficiency
1	KH_2PO_4 ..	1·088	..	..	..
2	KNO_3 ..	4·040	..	4·848	..
3	$Ca(NO_3)_2 \cdot 4H_2O$ ..	9·440	..	7·552	9·440
4	$MgSO_4 \cdot 7H_2O$ ..	3·936	3·936	3·936	3·936
5	K_2SO_4 ..	..	3·480	..	..
6	$Ca(H_2PO_4)H_2O$ ..	..	1·012	..	1·012
7	$CaSO_4 \cdot 2H_2O$ ..	..	2·752	..	..
8	H_3BO_3 ..	0·0228	0·0228	0·0228	0·0228
9	$MnCl_2 \cdot 4H_2O$ ..	0·0144	0·0144	0·0144	0·0144
10	$ZnSO_4 \cdot 7H_2O$ ..	0·0016	0·0016	0·0016	0·0016
11	$CuSO_4 \cdot 5H_2O$ ..	0·0006	0·0006	0·0006	0·0006
12	$H_2Mo\ O_4H_2O$ ..	0·0007	0·0007	0·0007	0·0007
13	Iron tartrate ..	0·0400	0·0400	0·0400	0·0400

* The figures indicate quantity of salts supplied per pot by the four Hoagland's nutrient solutions.

The nutrients were dissolved in eight litres of distilled water and supplied in two instalments of (1) five litres soon after planting and (2) three litres

a month later. Drainage holes in the pots were sealed so as to avoid leaching out of nutrients. Only requisite quantity of tap water was used for irrigation when needed. Care was taken to see that plants did not suffer from water-logging. Particular attention was paid to this aspect during the monsoon.

Twenty-five cultures were maintained for each variety and for each of the above four conditions of nutrition in all making up a total of $25 \times 5 \times 4$ or 500 cultures. Detailed studies on growth and other physiological characters would be reported separately. Only respiration studies under these conditions are presented here.

Towards maturity stage (250 days) samples of successive leaves were taken from the main shoot of each variety grown under four distinct conditions of nutrition. Each leaf was detached from the plant and cut into six inches long pieces. These were kept in the laboratory for 18 hours with their lower ends dipping in water. Rate of respiration of individual leaf samples was determined by Blackman's continuous current method with baryta as an absorbent. In all cases, carbon dioxide evolved was expressed in mgm. per hour per 10 grams fresh weight of leaves. Study included the simultaneous analysis of effect of five varieties, four nutritional conditions and five leaf ages on respiration. In all, it involved a study of one hundred combinations of these factors on leaf respiration under atmospheric oxygen and 30° C. bath temperature. Individual effects of these factors were statistically analysed by analysis of variance. Summary tables giving information on the effect of these factors on respiration are presented in the text along with singnificant differences at 5% level.

Experimental Results

A. *Effect of nitrogen deficiency on respiration of leaves.*—The effect of nitrogen deficiency differs characteristically in different varieties. In Co 453 all the five leaves from apex downwards showed consistently higher respiration in nitrogen-deficient culture. POJ 2878 also showed similar tendencies. Fourth leaf in this variety did not exhibit marked differences in respiration, when picked up from either the nitrogen-deficient or complete nutrient plant. In Co 421 respiration of first, second and third leaves from top was subnormal while that of fourth and the fifth was supernormal. There was no tendency of declining respiratory activity with advance in age in the nitrogen-deficient plants. Older leaves in complete nutrient media, on the contrary, exhibited decline in respiration. In Co 205, respiration of first, fourth and fifth leaf was supernormal in

deficient plants; slight subnormality was evident in the second and the third leaves. Respiration in complete nutrient plant rose to a high value in the third leaf and subsequently declined with advance in age. In Rheora respiration of the first, second and fourth leaf was super-normal and that of the third and the fifth was subnormal in nitrogen-deficient plants. In general, majority of leaves in Co 453 and POJ 2878 showed distinctly higher respiration in deficient plants. In other varieties, the effects were not so characteristic (Fig. 1).

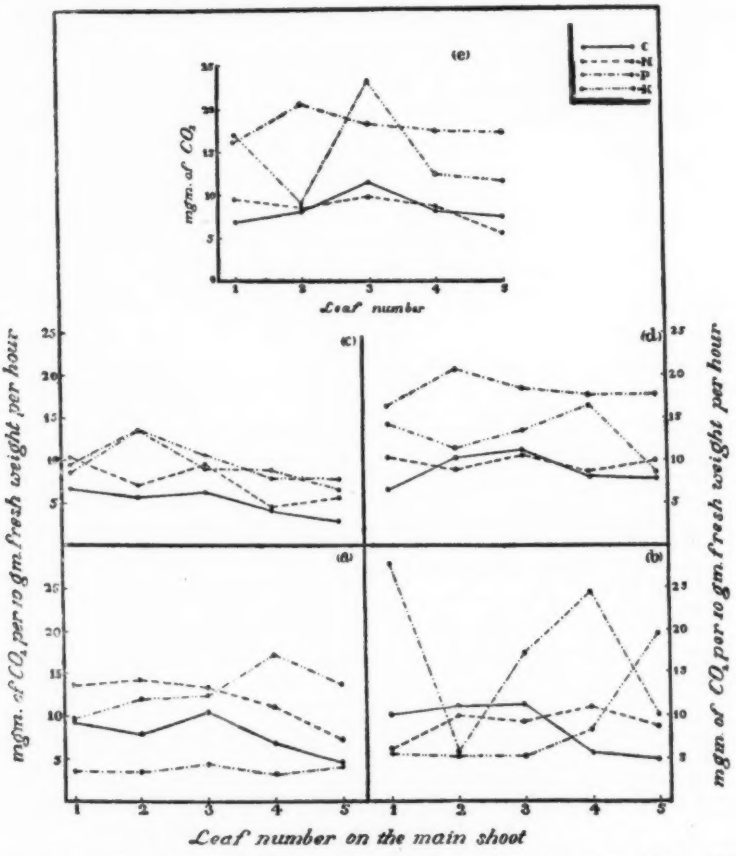

FIG. 1. Respiration of Leaves of (*a*) Co 453, (*b*) Co 421, (*c*) P.O.J. 2878, (*d*) Co 205 and (*e*) Rheora under difierent conditions of nutrition

B. *Effect of phosphorus deficiency on respiration of leaves.*—In contrast to nitrogen deficiency, absence of phosphorus caused higher respiration

rate of Co 453, POJ 2878, Co 205 and Rheora. Supernormal rates in all these four varieties were recorded in leaves of successive stages of development. In Co 421 respiration of first, second, and third leaves on phosphorus deficient plants was subnormal; that of fourth and fifth was supernormal. Age also showed distinct effects in as much as the third leaf stage was the critical stage upto which respiration of leaves on complete nutrient plants reached a high level; further advance in age lowered respiration of the leaves. Age effect in phosphorus deficient plants varied with varieties. No consistency in respiration was recorded in phosphorus deficient plants.

C. *Effect of potassium deficiency on respiration of leaves*.—Potassium deficiency in POJ 2878 and Rheora showed supernormal respiration rate of all leaves. In Co 453, on the contrary, subnormal respiration of all the leaves was noted in deficient cultures. In Co 421 and Co 205, all leaves except the second, exhibited higher respiration in deficient plants than similar leaves on the complete nutrient plants. In the complete nutrient plants, third leaf stage was the most critical upto which respiration of leaves increased and beyond which it fell with further advance in age. No such effects were noted on the potassium deficient plants in response to the stage of leaf development.

D. *Average respiration of leaves in relation to varieties, nutrients and age*.—Statistical analysis of the observations recorded above has shown that of the three factors the effect of which was under study, only nutrition showed a significant response. The effect of age and variety was insignificant (Table 1 *a*). Interactions between variety and age and between nutrition and age were equally insignificant. Of the first order interaction, variety × nutrition only showed significant effects.

Of the different conditions of nutrition provided to this crop, average leaf respiration in phosphorus and potassium deficient plants was significantly higher than similar values on complete nutrition or nitrogen-deficient plants. Differences in respiration of phosphorus deficient and potassium deficient plants were insignificant. Nitrogen deficiency did not show significant increases in respiration over that of complete nutrient plants. Relatively, phosphorus and potassium deficiency both were helpful in inducing significantly supernormal respiration rate of leaves (Table 1 *b* and *c*).

Of the five varieties, Rheora showed the highest respiration which was significantly higher than the respiration rate of Co 453 and POJ 2878. Differences between Co 421, Co 205 and Rheora were, however, insignificant. As a class these three varieties evinced higher respiration than the remaining two.

TABLE 1

Respiration of leaves in relation to varieties, nutrients and age

(*a*) Analysis of Variance

Due to	Degree of freedom	Sum of squares	Mean sum of squares	Ratio of variance	F at 5%
Age ..	4	80·0	20·0	1·25	5·70
Nutrient ..	3	430·0	143·3	9·01	8·58
Variety ..	4	281·3	70·3	4·42	5·70
Variety × Age ..	16	128·8	8·0	0·50	2·13
Variety × Nutrient ..	12	564·6	47·0	2·95	2·40
Nutrient × Age ..	12	71·6	5·9	0·37	2·40
Error ..	48	764·9	15·9	..	
Total ..	99	2321·2	..		

Standard error/Observation = $\frac{\sigma}{n}$ ± 3·98.

(*b*) Interaction of Variety × Nutrient

Varieties \ Nutrients	Complete nutrition	Nitrogen deficiency	Phosphorus deficiency	Potassium deficiency	Mean of 20
Co 453 ..	7·76	11·85	3·69	12·69	8·99
Co 421 ..	8·52	9·09	17·77	8·78	10·89
POJ 2878 ..	5·13	7·72	9·49	9·64	7·90
Co 205 ..	8·77	9·66	15·18	12·69	11·57
Rheora ..	8·01	8·45	18·50	14·68	12·40
Mean of 25 ..	7·72	9·27	12·71	11·69	..

Significant difference at 5% for Mean of 20 ..... 2·46
„ „ 25 ..... 2·22
„ „ 5 ..... 5·02

(*c*) Interaction of Age × Nutrient

Leaf Age \ Nutrition	Complete Nutrition	Nitrogen deficiency	Phosphorus deficiency	Potassium deficiency	Mean of 20
1 ..	7·90	9·93	14·46	10·90	10·80
2 ..	8·55	9·76	10·63	10·23	9·79
3 ..	10·05	10·52	13·13	12·71	11·61
4 ..	6·48	8·68	14·55	12·37	10·52
5 ..	5·59	7·48	10·76	12·26	9·02
Mean of 25 ..	7·72	9·27	12·71	11·69	..

(*d*) Interaction of Variety × Age

Leaf Age \ Varieties	Co 453	Co 421	POJ 2878	Co 205	Rheora	Mean of 20
1 ..	8·95	12·36	8·70	11·60	12·39	10·80
2 ..	9·35	8·14	9·95	9·96	11·57	9·79
3 ..	9·70	10·76	8·83	12·99	15·79	11·61
4 ..	9·50	12·32	6·31	12·87	11·59	10·52
5 ..	7·48	10·86	5·68	10·45	10·63	9·02
Mean of 20 ..	8·99	10·99	7·90	11·57	12·40	..

Significant difference at 5% for Mean of 25 ..... 2·22
„ „ 20 ..... 2·46
„ „ 5 ..... 5·02
„ „ 4 ..... 5·41

So far as the age effect is concerned, the third leaf tended to exhibit high respiration; this was significantly higher than the respiration of fifth leaf, which showed lowest respiration. Differences in respiration of first, second, third, and fourth leaf was statistically insignificant. It, therefore, appears that only after the leaf has passed the fourth leaf stage of development that respiration showed a decline (Table 1 *d*).

Discussion

Data recorded in the previous pages have shown certain outstanding effects of nitrogen, phosphorus and potassium deficiencies on respiration. Average figures for different conditions of leaf development and varieties indicate that deficiency of nitrogen does not bring about any significant variation in respiratory activity of successive leaves on the main shoot (Table 1 *c*). Overall leaf age values also indicate that varieties amongst themselves, again, do not exhibit significant differences, the complete nutrient and nitrogen-deficient plants appear alike in respiratory activity. There is, therefore, no question of subnormality in leaf respiration in nitrogen-deficient canes, though similar phenomenon has been noted in barley.[5,6] This anomaly is explained on account of higher sucrose and carbohydrates that accummulate in cane under conditions of nitrogen starvation.[26] Depression, if any, caused by absence of nitrogen under such conditions is made good by high sucrose in plants. Obviously, therefore, two opposing tendencies, *viz.*, that of nitrogen deficiency to depress respiration and of high carbohydrate to accelerate it,[30] appear to determine respiratory response of sugarcane under conditions of nitrogen starvation.

Comparative figures for barley indicate that nitrogen deficiency causes subnormality in respiration[4] and is often accompanied by high sugar and low nitrogen[6] and large total sugars.[5] Other changes include high sucrose/reducing sugars, high assimilation, low protein synthesis and low meristematic activity in nitrogen-starved barley.[5] In other crops, nitrogen in lower doses causes high sucrose/reducing sugars and starch to hexose ratio[16] and increases reducing sugars, sucrose and poly-saccharides.[10,11,12] Subnormality in nitrogen-deficient plants is, therefore, linked with favourable variations in carbohydrates and nitrogen fractions. And cases are not unlikely where subnormality in absence of nitrogen may be substantially counter-balanced by supernormality in respiration caused by richer respirable substrate in deficient plants.

Phosphorus deficiency in the present investigations, results in supernormal respiration. This is characteristically noted in the first, fourth and fifth leaf on the main shoot (Table 1 *c*). In the second and third leaves, increased respiration in the phosphorus deficient plants does not attain the level of significance. Similar supernormal respiration is noted in varieties Co 421, Co 205 and Rheora, where the increases due to phosphorus deficiency are significant. In other two varieties Co 453 and POJ 2878 differences between complete nutrient and phosphorus deficient plants are insignificant. These results are in contrast to the findings on barley plants[4] where the

effects of phosphorus deficiency were noted to be normal. In phosphorus deficient barley, normal respiration is associated with medium sucrose/reducing sugar ratio, high reducing sugars and high amide/total amino nitrogen.[4] In another instance, phosphorus deficiency is claimed to be associated with low assimilation, low sugar content, low respiration, low protein and high translocation.[5] In contrast to these, phosphorus deficiency is noted to increase percentage of starch and total carbohydrates[13,25] and to result in high reducing sugars, high sucrose and high total carbohydrates.[12,15] Consistently higher rates of respiration in the three varieties Co 421, Co 205 and Rheora at maturity and in the three leaves on the main shoot appear correlated with favourable variations in total carbohydrates in phosphorus deficient plants. Why sugarcane leaves in the present instance show supernormal respiration in contrast to normal[4] or low respiration[5] of phosphorus deficient, barley cannot be said with certainty at present.

Potassium deficiency is also noted to induce supernormal respiration in sugarcane. Differences in respiration of fourth and fifth leaf from complete nutrient and potassium deficient plants are highly significant (Table 1 *c*). Amongst the varieties, potassium deficient Rheora shows significantly higher respiration than plants of complete nutrition. Average figures for all the varieties, again, point out supernormal respiration under potassium deficiency (Table 1 *b*). Respiration of potassium deficient plants is significantly higher than the average respiration for either the nitrogen deficient or complete nutrition canes, but does not differ significantly from phosphorus deficient plants. In barley, similar supernormal rates were recorded under potassium deficiency,[4, 5, 6] and were accounted for, on the basis of substantial increase in amino nitrogen. In sugarcane, potassium deficiency has an unfavourable effect on sucrose and total carbohydrates.[26] Reasons for supernormality have, therefore, to be traced to other respirable substrates. The possibility is that in the absence of sufficient carbohydrate in potassium-deficient plants, nitrogen in the plant may not be fully utilized in building up processes and may, therefore, be in a state of relative abundance. Favourable changes in nitrogen fractions if established, might help in elucidating the physiological causes of supernormality in potassium deficient canes. Comparative effects of phosphorus and potassium deficieny do not lead us anywhere to a proper explanation of the causes leading to supernormality in respiration. Possibility of stimulation as a result of high carbohydrates in phosphorus deficient plants and of higher nitrogen compounds in potassium deficient canes is visualized.[23] Further light on this aspect would be thrown in subsequent contributions.

Effect of age on respiration does not seem to be significant (Table 1 *a*). None of the interactions with age have been found to be significant. Average figures for leaves of different ages in different varieties, again, seem to be insignificant. Respiration beyond the third leaf stage shows a definite fall with age, which is significantly low in the case of fifth leaf. In leaves of younger age, there seems to be no significant differences. At maturity stage of sugarcane, therefore, age of individual leaves does not alter respiration significantly except in leaves of advanced age. The effect of age seems to be complicated by other factors and it is not always possible to separate effects attributed to age from changes due to other causes, *viz.*, nutrition.[22] For instance in sugarcane[13, 25, 26] nitrogen and potassium are known to affect respiration only during early periods. Phosphorus, on the contrary, augments respiration in the second half and diminishes it in the first half of the life-cycle. How far nutrition and age effects on respiration differ with the stage in the lifecycle will be described in a separate communication.

Comparative figures for the varieties show that Rheora has the highest average respiration and POJ 2878, the least. Differences between the two are statistically significant. Co 453 and POJ 2878 have relatively low respiration rates while Co 421, Co 205 and Rheora exhibit high respiration. Differences amongst the individual varieties of each of these two groups, however, are not significant. What is the cause of high respiration of Co 205 and Rheora as compared to Co 453 and POJ 2878 cannot be indicated with certainty till further information on compositional variations are available under these conditions of nutrition.

Summary

The paper narrates the results of an experimental enquiry on respiration rate of sugarcane leaves of different stages of development under four conditions of nutrition, *viz.*, complete nutrition, nitrogen deficiency, phosphorus deficiency and potassium deficiency. The experiments were conducted at maturity stage on five varieties of sugarcane, *e.g.*, Co 453, Co 421, POJ 2878, Co 205 and Rheora grown in sand in pots.

Co 205 and Rheora show high leaf respiration as compared with Co 453 and POJ 2878, having significantly lower respiration rates. No differences in leaf respiration upto the third or fourth leaf stage are noted; beyond this the greater the stage of leaf development, the lower is the respiration of leaves.

Nutritional conditions bring about considerable changes in respiration of leaves. Thus nitrogen deficiency shows normal leaf respiration at

maturity as compared with either phosphorus or potassium deficiency, each of which exhibit supernormality in respiration.

Variations in respiration rate under these conditions appear to be determined by changes in chemical composition of leaves, particularly nitrogen and carbohydrate fractions. Observed differences in respiration are discussed in the light of such changes.

Literature Cited

1. Berry, W. E. and Steward, F. C. .. "The absorption and accummulation of solutes by living plant cells. VI—The absorption of potassium bromide from dilute solution by tissues from various plant storage organs," *Ann. Bot.*, 1934, **48** (190), 395–410.
2. Chaterjee, U. N. .. "Studies on the effects of phosphates on respiration of green leaves. 1. *Eugenia jambolana* 2. *Allium tuberosum*," *Bull. Acad. Sci., United Provinces of Agra and Oudh*, 1933, **2** (3), 179–92.
3. Eijh, M. Van .. "Einfluss der Salzanfnahne auf die Wurzelatmung bei aster tripolium. *K. Acad. Wetenschap Amsterdam*," *Proc. Sect. Sci.*, 1938, **41** (10), 1115–21.
4. Gregory, F. G. and Richards, F. J. "Physiological studies in plant nutrition 1. The effect of manurial deficiency on respiration and assimi, lation rate in barley," *Ann. Bot.*, 1929, **43** (169), 119–61.
5. ——— and Baptiste, E. C. D. .. "Physiological studies in plant nutrition. V. Carbohydrate metabolism in relation to nutrient deficiency and to age in leaves of barley," *ibid.*, 1936-**50** (199), 579–619.
6. ——— and Sen, P. K. .. "Physiological studies in plant nutrition. VI. The relation of respiration rate to the carbohydrate and nitrogen metabolism of the barley leaves as determined by nitrogen and potassium deficiency," *ibid.*, 1937. **1** (3), 521–61.
7. Hamer, K. C. .. "Effects of nitrogen supply on rates of respiration and photosynthesis of plants," *Bot. Gaz.*, 1936, **97** (4), 744–64.
8. Jones, Winston W. .. "Respiration and metabolism in etiolated wheat seedlings as influenced by phosphorus nutrition," *Plant Physiol.*, 1936, **11** (3), 565- 82.
9. Kerr, H. W. .. "Factors influencing sugar content of cane," *Cone Growers" Quart. Bull.*, 1934, 133.
10. Kraus, E. J. and Kraybill, H. R. "Vegetation and reproduction with special reference to the tomato," *Oregon. Agr. Exp. Sta. Bull.*, 1918, 149.
11. Kraybill, H. R. and Smith, T. O. "Plant metabolism studies. New Hampshire," *Agric. Exp. Sta., Ann. Rept. Bull.*, 1924, 212.

12. Kraybill, H. R. .. "Plant metabolism studies as an aid in determining fertilizer requirements," *I. Indus. and Eng. Chem.* 1930, **22**, 275.

13. Lal, K. N. and Pathak, H. S. .. "Studies in crop physiology. Effects of fertilizer deficiency and sufficiency upon growth and development of sugarcane," *Journ. Ind. Bot. Soc.*, 1948, **27** (1).

14. ——— and Shankarji Shrivastava "Studies in crop physiology. Nutrient effects upon development and vegetative vigour of sugarcane," *Proc. Ind. Acad. Sci.*, B, 1949, **29** (3), 109–28.

15. Mac. Gillivray, J. H. .. "Effects of phosphorus on the composition of the tomato plant," *Journ. Agric. Res.*, 1927, **34**, 97.

16. Orcutt and Wilson .. "Effect of nitrate nitrogen on the carbohydrate metabolism of inoculated soyabeans," *Soil. Sci.*, 1935, **39**, 289.

17. Overholser, E. H. and Claypool, L. L. .. "The relation of fertilizers to respiration and certain physical properties of strawberries," *Proc. Amer. Soc. Hort. Sci.*, 1932, **28**, 220–24.

18. Petrie, A. H. K. .. "The intake of ions by the plant and its relation to the respiration of the root," *Aus. Journ. Exp. Biol. and Med. Sci.*, 1933, **11** (1), 25–34.

19. ——— and Williams, R. F. .. "Physiological ontogeny in plants and its relation to nutrition. V. The effect of nitrogen and phosphorus supplies on the respiration rate of the leaves," *Aus. Journ. Exp. Biol. and Med. Sci.*, 1938, **16** (4), 347–60.

20. Phillips, T. G., Smith, T. O. and Dearborn, R. B. .. "The effect of potassium deficiency on the composition of tomato plant," *New Hampshire Agric, Exp. Sta. Tech, Bull.*, 1934, 59.

21. Richards, F. J. .. "Physiological studies in plant nutrition. III. Further studies of the effect of potash deficiency on the rate of respiration in the leaves of barley," *Ann. Bot.*, 1932, **46**, 367.

22. ——— .. "On the use of simultaneous observations on successive leaves for the study of physiological changes in relation to leaf age," *ibid.*, 1934, **48** (190), 497–504.

23. ——— and Templeman, W. G. .. "Physiological studies in plant nutrition IV. The relation of manurial deficiencies to nitrogen metabolism of barley leaves," *ibid.*, 1936, **1**, 367

24. ——— .. "Physiological studies in plant nutrition. VIII. The relation of respiration rate to the carbohydrate and nitrogen metabolism of the barley leaf as determined by phosphorus and nitrogen supply," *ibid.*, 1938, **2** (6), 491–534.

25. Singh, B. N. .. "Progress reports of researches on physiology of wheat and sugarcane for 1937-38, 1938-40, 1940-42," *Ind. Council of Agric. Res.*, New Delhi.

26. Singh, B. N. .. "Age fertilizer effects upon certain aspects of the physiology and chemistry of sugarcane," *Proc. Ind. Acad. Sci.*, B, 1941, **13** (5).

27. ———— .. "Physiological effects of deficiency or excess of added fertilizers upon growth characters, carbohydrate metabolism and yield and juice quality of sugarcane," *ibid.*, 1941, **15** (4).

28. Steward, F. C. and Berry, W. C. .. "The absorption and accumulation of solutes by living plant cells. VII. The time factor in the respiration and salt absorption of Jerusalem artichoke tissue (*Helianthus tuberosus*) with observations on ionic interchange," *Journ. Exp. Biol.*, 1934, **11** (2), 103–119.

29. Stocklasa, J. .. "Ist das Kalium an der Eiweissynthese in der. Pflanzenzelle beteiligt?" *Biochem. Zeitschr.*, 1916, **73**, 107.

30. White, H. L. .. "The interaction of factors in the growth of Lemna, VII. The effect of potassium on growth and multiplication," *Ann. Bot.*, 1936, **1**, 175–96.

31. ——— and Templeman, W. G. .. "The interaction of factors in the growth of Lemna, X. The interaction of nitrogen and light intensity in relation to respiration," *ibid.*, 1937, **2** (1), 191–204.

ON THE REPRODUCTIVE ORGANS OF *PISIONE REMOTA* (SOUTHERN), TOGETHER WITH A REVIEW OF THE FAMILY PISIONIDÆ (POLYCHÆTA)*

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Introductory

An aberrant family of small-sized polychætes with uncertain affinities, the Pisionidæ comprises species which reveal a degree of specialisation that is generally rare in the group. The occurrence of these interesting polychætes in the inter-tidal zone of the Madras beach has been reported in a series of contributions (Aiyar and Alikunhi, 1940; Alikunhi, 1941, 1947), and the anatomical studies of the three new species, *Pisionidens indica*, *Pisione gopalai* and *P. complexa* have now rendered it possible to re-examine the affinities of the family and revise the family and generic characters.

The occurrence of the Irish species *Pisione remota* (Southern) also in the Madras beach has enabled a detailed study of its reproductive structures, which has hitherto been lacking. An account of the same is set forth in the succeeding pages to facilitate consideration of the affinities of the family.

Pisione remota (Southern)

Originally described by Southern (1914) from the Irish waters as the type of a new genus *Prægeria*, it sufficiently resembles the type of *Pisione*—

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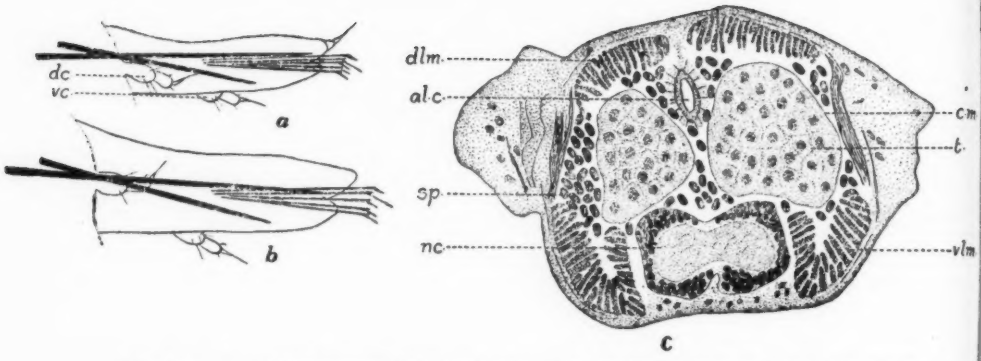
P. ærstedii Grube—that Hartman (1939) considered its retention as a separate genus unnecessary. A comparative study of the Madras species, *Pisione gopalai* which was first assigned to Southern's genus *Prægeria* (Alikunhi, 1941) and of *P. complexa*, with the type *P. ærstedii* led the present writer also to agree with Hartman's views (Alikunhi, 1947). According to the latter *Pisione germanica* (Augener, 1924) from the North Sea agrees with the description of *Prægeria remota* Southern from Ireland. Smith (1932) has found *P. remota* occurring in large numbers in the in-fauna of the shell gravel deposits of the Eddystone grounds, Plymouth. Thus, while the species does not appear to have been recorded from outside Europe, its occurrence in the purely tropical environment at Madras is of great interest as it demonstrates the successful adaptation of a species to extremes of environments.

Three species, *Pisione gopalai*, *P. complexa* and *P. remota* occur in the inter-tidal zone of the Madras beach. As is common in the sandy beach, they exhibit a degree of zonation in their occurrence, though some overlapping of the different zones does take place. *P. gopalai* is found in the fine sand at about half tide level, together with *Pisionidens indica*; while in the coarser sand, nearer to the low tide level, *P. complexa* abounds. *P. remota* confines itself to the rather coarse sand near low water level and is never found in the higher zone in which *P. gopalai* occurrs in large numbers. Rarely, however, all the three species may be found in the same sample of sand taken from the low water level.

Two mature female specimens of *P. remota* were obtained (on 17–9–1940) from Sandy Point', Krusadai Island, in the Gulf of Mannar, in similar substratum and zone as found at Madras.

As in the allied species, a rich development of hypodermal adhesive glands is characteristic of the Madras specimens. This is, perhaps, of extreme importance since the particular environment at low tide level, being subject to the churning and shifting action of the incessant waves, ever holds out the threat of dislocating them from their natural niches. Southern's descriptions of the external characters is found to be generally accurate when compared to the Madras specimens. In the latter, however, the tip of the parapodial lobe in the anterior segments is distinctly pointed, carrying a minute fillet which terminates in a fine point (Fig. 1 *a*). This fillet is absent in the posterior parapodia (Fig. 1 *b*). The setigerous support consists of two acicula and five setæ. In the Irish specimens a second simple seta appears in every foot, beginning from the tenth. Such a seta is absent in the Madras specimens, which, however, have in every foot, an additional compound seta with a long terminal blade, as in *P. gopalai* and *P. complexa*.



TEXT-FIG. 1. *Pisione remota* (Southern).—(*a*) First setigerous foot, × 135; (*b*) Tenth setigerous foot, × 135. (*c*) Transverse section through the anterior half of the testes-bearing segment of a male worm. Paired nature of the testes and the membraneous structure (?) delimiting the same, are distinct, × 200.

al.c. Alimentary canal; *cm.* Covering membrane (?); *dc.* Dorsal cirrus; *dlm.* Dorsal longitudinal muscle; *nc.* Nerve cord; *sp.* Sperm; *t.* Testis; *vc.* Ventral cirrus; *vlm.* Ventral longitudinal muscle.

REPRODUCTIVE ORGANS

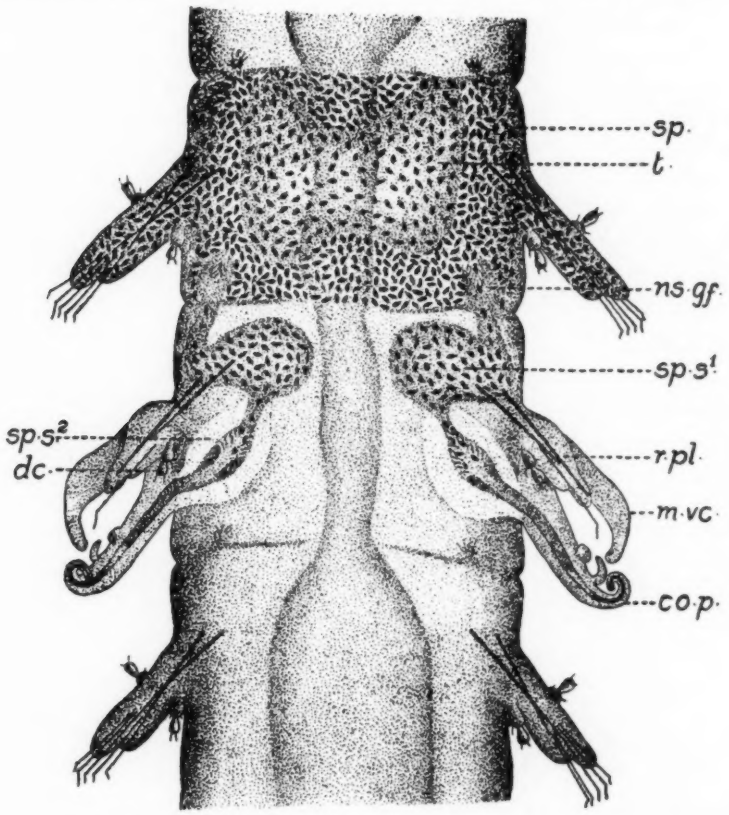
Male: Testis.—The gonads are generally restricted to the middle segments of the body. The male usually has only a single pair of testes. In a specimen with 27 setigerous segments, a pair of testes were present in the 17th segment. A thin covering membrane appears to envelope the testis (Fig. 1 *c*). When ripe, the two testes coalesce into a single mass, as is found in the other species of the genus. In the testes-bearing segment the gut is generally pushed dorsad and the longitudinal muscles are thin and reduced.

Sperm-sacs.—The testes are followed by a single pair of sperm-sacs which in the majority of specimens are situated in the 19th segment, though they may occur in any one of the segments from the 16th to the 20th. Occasionally two pairs of sperm-sacs and copulatory organs have been found developed in two consecutive segments. For example, in a specimen with 32 setigerous segments, sperm-sacs were developed in the 16th and 17th segments, while, the testes were present in the 15th and 16th.

Genital funnels which develop in association with the nephridia in the testes-bearing segments, resemble those of *P. gopalai* and *P. complexa* in their size, shape and disposition. The nephridial swelling appears small and inconspicuous, compared to the large ciliated organ that is grafted on to it (Fig. 3*a*). The nephridial duct behind the septum is enlarged into the sperm-sac. Proximally it is thin-walled and spacious, while, distally it is muscular and thick-walled, with narrow lumen (Fig. 3 *a*). The inner lining

epithelium at the distal portion is thin anteriorly, while posteriorly the cells become columnar, highly protoplasmic and provided with refringent granules. Sperms are generally stored in the proximal dilatation. The terminal portion of the nephridial duct—the efferent duct of the sperm-sac—now enters the copulatory organ. The entire modified nephridial duct, except the last $\frac{1}{3}$ which is cutinised, is richly ciliated internally.

Copulatory Organs.—In the segment containing the sperm-sacs, the parapodial lobe, unlike the condition in *P. gopalai* and *P. complexa*, remains



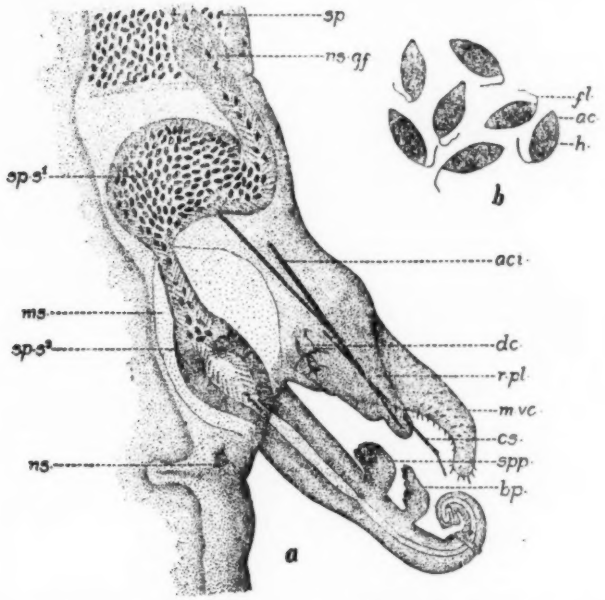
TEXT-FIG. 2. *Pisione remota* (Southern).—Genital segments of a ripe male showing the general arrangement of the reproductive structures. Drawn from life; specimen pressed under the cover glass, × 100.

co.p. Copulatory process; *m.vc.* Modified ventral cirrus; *ns.gf.* Nephridial swelling and associated genital funnel. *r.pl.* Reduced parapodial lobe; *sp.s*¹. First dilatation of sperm-sac; *sp.s*². Second dilatation of sperm-sac. (Other letters as in previous figures.)

distinct though rudimentary and in the form of a conically pointed structure, broad at base and indented behind the tip (Fig. 3 *a*). It carries two acicula and one compound seta which is identical with the one having the longer terminal blade in the unmodified parapodium. The dorsal cirrus remains unchanged. The ventral cirrus is highly modified and is in the form of a stout finger-like process, broad at the base and curved and tapering at the tip which is provided with stiff sensory hairs. The copulatory apparatus consists of this modified ventral cirrus and an elongated process—the copulatory organ proper—developed ventrally to it. The latter is a long, curved, partially retractile structure, tapering to the tip and carries the efferent duct of the sperm-sac. At about its middle is a hood-like process with the tip beset with a number of minute cuticular projections (Fig. 3 *a*), corresponding to the spinous papilla in the copulatory organ in the other two species. Beyond this, is a knife blade-like appendage in the form of a recurved hook with the inner edge cutinised and raised into minute projections. The distal extremity of the copulatory process is curved inwards and at the beginning of the curve there is a slender elongated process with a minute finger-like papilla on one side. The duct of the sperm-sac opens at the tip of the copulatory organ which is blunt and cutinised on the outside (Fig. 3 *a*).

A comparison of the copulatory apparatus in the three species of the genus, *viz.*, *P. gopalai*, *P. complexa* and *P. remota*, shows that even though the structure conforms to a common plan, there are important specific differences. The copulatory organ proper has been shown to be a structure formed *de novo* from the body wall near the base of the ventral cirrus (Alikunhi, 1947). The position of the copulatory organ in *P. remota* clearly supports this view; and further evidence that this mode of formation of the copulatory organ is, probably, the general rule in the Pisionidæ, is forthcoming from a detailed study of the development and differentiation of similar but more complicated structures in *Pisionidens indica* (Alikunhi, 1948). In *P. gopalai*, however, there is an extreme reduction of the parapodia and the ventral cirri of the segment in which the sperm sacs are situated, so that the adult structure is simple and entire. It is interesting that unlike the condition in the other two species, the copulatory structure is curved away from the body in *P. remota* (Figs. 2, 3 *a*). The shape of the modified ventral cirrus, the undivided nature of the tip of the copulatory hook, and the position and structure of the accessory processes on the copulatory organ are all features in which *P. remota* markedly differs from *P. complexa*.

Sperms.—The sperms are non-motile. When mature they are liberated into the cœlom, but do not pass into the neighbouring segments (Fig. 2). Each sperm measures about 11 to 14 microns in length. The tail or flagellum



TEXT-FIG. 3. *Pisione remota* (Southern).—(*a*) Magnified view of the sperm-sac and copulatory organ. Drawn from life ; specimen pressed under cover glass, × 135. (*b*) Sperms from the male. From a fresh preparation, × 600.

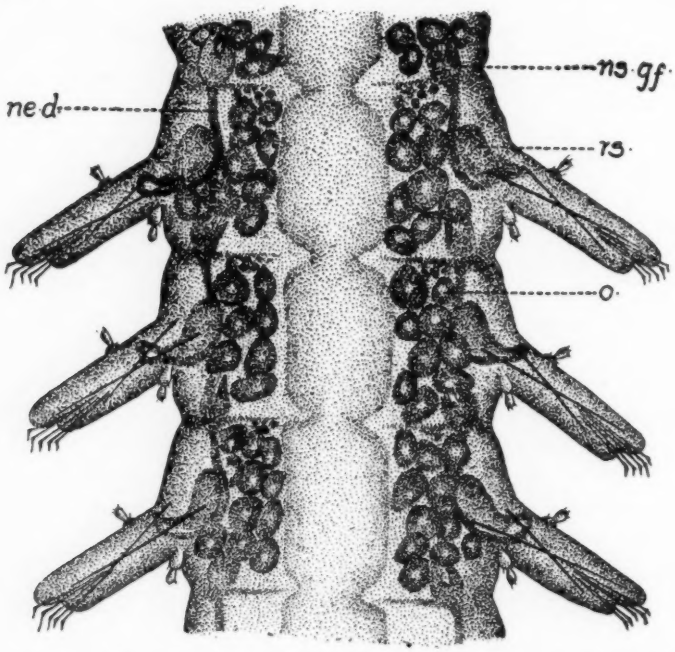
ac. Acrosome ; *aci.* Aciculum ; *bp.* Blade-like papilla ; *cs.* Compound seta ; *fl.* Flagellum ; *h.* Head ; *ms.* Muscular sheath ; *ns.* Nephridial swelling ; *spp.* Spinous papilla. (Other letters as in previous figures.)

is short and slender and is often curved forwards along the side of the head (Fig. 3 *b*). The head portion is thick and spindle-shaped, with a minute pointed acrosome in front and an inconspicuous neck behind. From the acrosome to the neck it measures about 7·5 to 9 microns. In sections of the worm the nuclear portion of the sperm takes deep stain, while the acrosome and neck cannot be made out.

Female.—Ova are developed in the middle and posterior segments. In a specimen with 35 setigerous segments ova were present in the 18th to the 32nd segment, while in another with 52 segments (Krusadai specimen) they were found in the 20th to the 46th. Ova in one segment do not freely pass into the neighbouring ones. They are large and greenish in colour.

Paired genital funnels are developed in all the ova-bearing segments, in association with the nephridia which open to the exterior in the succeeding segment. Following each pair of ovary a pair of receptacula seminis

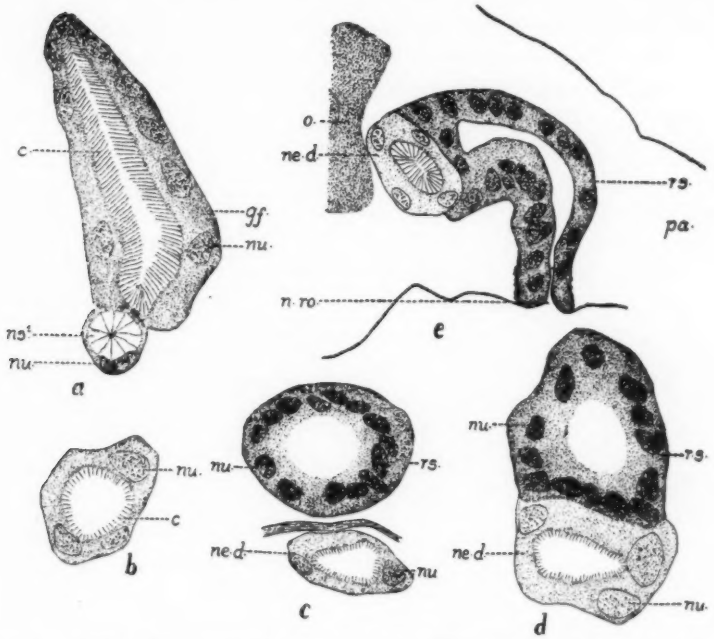
are developed in association with the nephridial duct (Fig. 4); *e.g.*, in a specimen with 52 setigerous segments and 27 pairs of ovaries, situated in segments 20 to 46, genital funnels were present in all the ova-bearing segments, while



TEXT-FIG. 4. *Pisione remota* (Southern). Three consecutive genital segments of a ripe female, showing the characteristic distribution of the reproductive organs. Drawn from life, × 100.

ne.d. Nephridial duct; *o.* Ovum; *rs.* Receptaculum seminis. (Other letters as in previous figures.)

receptacula seminis were developed in segments 21 to 47. As in the male, in the ova-bearing segments a large genital funnel gets grafted on to the dorso-lateral aspect of the nephrdial swelling. In sections the two structures could be distinguished by the nature of their cells and their different staining reaction (Fig. 5 *a*). Behind the septum the nephridial duct gets enlarged, has thick walls (Fig. 5 *b*), and running external to the oblique muscles assumes a position on the ventral aspect of the receptaculum seminis from which, though in intimate contact, it remains distinct (Fig. 5 *c–d*). Towards the external end of the duct of the receptaculum seminis it is joined by the nephridial duct and the short common duct so formed opens to the exterior ventrally at the parapodial base (Figs. 5 *e* and 6 *b*).



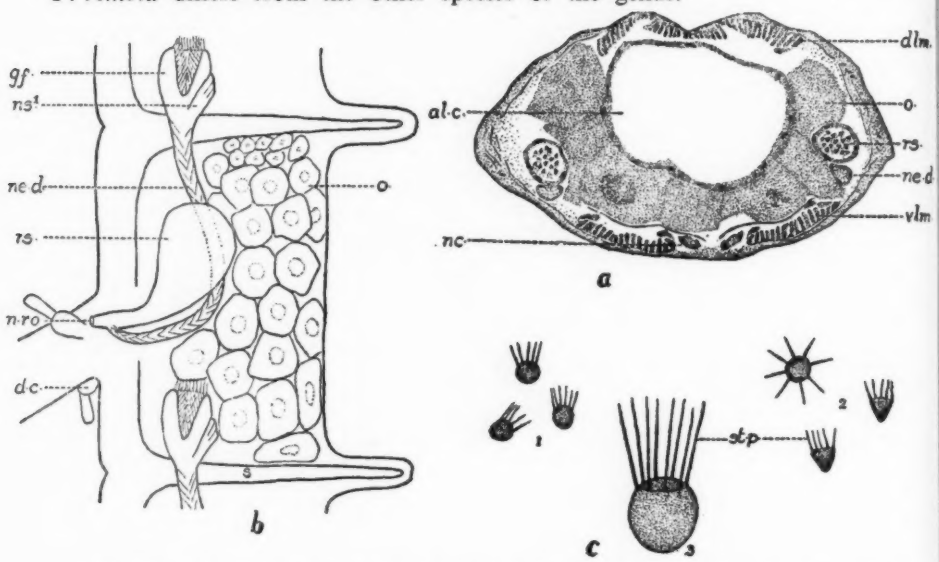
TEXT-FIG. 5 (*a*) *Pisione remota* (Southern).—(*a*) Transverse section of the nephridial swelling and the associated genital funnel of a female segment. The nephridial portion on the ventral aspect, is relatively inconspicuous, × 600. (*b*) Transverse section of the nephridial duct, in a female genital segment, immediately behind the anterior septum, × 600. (*c*) Transverse section through the closed end of the receptaculum seminis of a female (not fully mature). Nephridial duct is distinct, on the ventral aspect, × 600. (*d*) Transverse section through the middle of the receptaculum seminis of a female (not fully ripe). The nephridial duct is intimately apposed to the ventral wall of the receptaculum seminis, × 600. (*e*) Portion of a transverse section showing the external aperture of the receptaculum seminis ventrally, at the base of parapodial lobe, × 450.

c. Cilium; *gf*. Genital funnel; *n.ro*. Nephridio-receptacular opening; *ns*¹. Reduced nephridial swelling. *nu*. Nucleus; *pa*. Parapodium. (Other letters as in previous figures.)

Parapodia of the female segments are unmodified (Fig. 4). The receptacula seminis are thin-walled sacs occupying the lateral portions of the segmental compartment near the parapodial base (Fig. 6 *a*). In the ripe females they are invariably filled with sperms. The cells forming their walls are large and protoplasmic, with big deeply staining nuclei. A few circular muscle fibres are noticed near the external aperture of the receptacular duct (Fig. 5 *e*).

It is thus seen that a female genital segment in *P. remota* has a pair of ovaries, a pair of genital funnels in association with the nephridia and

a pair of receptacula seminis (Fig. 6 *b*), while, in *P. gopalai* and *P. complexa* the structures are not so crowded and one or more of these may be wanting in any particular genital segment. In the unmodified condition of the parapodia of the segments carrying the receptacula seminis and in the absence of any special structures at the nephridio-receptacular aperture, also, *P. remota* differs from the other species of the genus.



TEXT-FIG. 6. *Pisione remota* (Southern).—(*a*) Transverse section of a female genital segment showing the receptaculum seminis in the segmental chamber, × 180. (*b*) Diagram showing the relation between the nephridial duct and the duct of the receptaculum seminis. (*c*) Sperms from the receptaculum seminis ; (1) From a fresh preparation, × 600. (2) From section of the receptaculum seminis, × 600. (3) Diagram of a metamorphosed sperm.

st.p. Stiff processes. (Other letters as in previous figures.)

Sperms from the Receptaculum Seminis.—The invariable presence of sperms in the receptaculum seminis of the ripe female indicates copulation between the sexes, though the actual process has not been observed. Further, the sperms inside the receptaculum seminis are non-motile and different in structure from those present in the males. After transference to the receptaculum seminis they have lost their characteristic spindle-shaped structure and are found to have acquired a spherical shape with a circlet of stiff, tapering, fine processes, probably anteriorly (Fig. 6 c^1). Each sperm is provided with 5 to 8 such processes, the entire structure reminding one, of the characteristic zoospores of plants. After fixation in Bouin's fluid only a portion of its nucleus takes stain and this part is roughly in the form of a V,

Hæmatoxylin, following fixation in Flemming's fluid without acetic, gives a better picture of these sperms, the stiff processes taking moderate stain, when they appear as arising from the circumference of a disc (Fig. 6 c^2). There is no formation of spermatophores. The sperms have also undergone considerable reduction in size (Figs. 3 *b* and 6).

The spherical or disc-like portion of the metamorphosed sperm seems to represent the nucleus or the head, but it does not take uniform stain like the sperm nucleus in the male. The crown of processes, therefore, might have arisen either from the acrosome or from the neck region. Since in *P. gopalai* and *P. complexa* the sperms in the receptaculum seminis undergo modifications mainly at the anterior end, it is probable that in *P. remota* also it is the anterior acrosomal region that has been modified into the stiff processes, the reduced neck and flagellum probably being completely suppressed. These stiff, probably adhesive, processes are reminiscent of the structure of the decapod sperm. It is interesting to note in this connection that according to Riley (quoted by David Sharp, 1918) the spermatheca (receptaculum seminis) in *Pronuba* (Lepidoptera—Heterocera) contains some curious radiate bodies; while Godman and Salvin also describe similar bodies as existing in butterflies.

The characteristic reproductive structures and the remarkable metamorphosis of sperms in the pisionids studied, probably indicate certain ecological adjustments for ensuring propagation of the species. In polychætes in general, the gonads are very much diffused and in the majority of cases the genital elements are shed into the surrounding medium where fertilization takes place. In the inter-tidal zone, the prevailing extremes of conditions may not ensure the requisite meeting of the opposing elements, with the result that the minute cryptic forms like *Pisionidens*, *Pisione*, *Microphthalmus*, *Saccocirrus*, etc., have developed specialised accessory structures like sperm-sacs, copulatory organs and receptacula seminis which ensure the successful fertilization of the limited number of ova produced. In these forms ova get fertilized just before or at the point of extrusion. As a further step, the pisionid sperm undergoes metamorphosis and becomes adhesive in nature so that the slightest contact with the ovum will be sufficient to effect fertilization. The rigours of this remarkable inter-tidal zone have thus necessitated elaboration of wonderful structural adaptations that render life and propagation of these little denizens possible in the environment.

Genus *Pisione*—Revision of Characters

Existing accounts of the generic characters of *Pisione* have been based on descriptions of external features of *P. œrstedi* and *P. remota*, while, nothing

TABLE I

Statement of Distinguishing Characters of the Known Species of Pisione *Oersted*

Specific Features	*P. oerstedii*	*P. remota*	*P. gopalai*	*P. complexa*
Length	20 mm. to 40 mm.	4 mm. to 10 mm.	4 mm. to 10 mm.	10 mm. to 25 mm.
Number of body segments	125 to 160	25 to 52	20 to 50	70 to 100 or more
Buccal spines	Present	Long; 92–95 μ	65 μ long	74 μ long
Ventral cirrus of buccal parapodium	Globular	Flask-shaped	Globular	Globular
Ventral cirrus of first parapodium	Long	Long; about 90 μ	Short; about 40 μ	Short; about 56 μ
Anal segment	Semi-circular	Semi-circular	With conspicuous caudal glands	Semi-circular
Anal cirri	Two; long	Two; long, swollen at base	Two; long, not swollen at base	Two; long, swollen at base
Parapodia	Sub-biramous	Sub-biramous	Sub-biramous	Sub-biramous
Setigerous support ..	Two acicula and six setæ	Two acicula and five setæ	Two acicula and five setæ	Two acicula and five setæ
Hind lobes of brain extend to		4th setigerous segment	Posterior half of 3rd setigerous segment	Anterior half of 3rd setigerous segment
Jaws	Two pairs	Two pairs	Two pairs	Two pairs
Proboscis	Protrusible; with papillæ	Protrusible; with papillæ	Protrusible; with papillæ	Protrusible; with papillæ
Number of pairs of testes		*Always one*; confined to	*Always one*; often	

Number of pairs of testes ..	*Always one;* confined to one segment	*Always one;* often extending to the adjoining segment	*One to six* or more; each confined to one segment
Number of pairs of sperm-sacs ..	1 or 2; 2nd saccular dilatation inconspicuous	*Always one*; 2nd dilatation conspicuous	1 to 6 or more; 2nd dilatation absent
Number of pairs of genital funnels ♂	1 or 2	*Always one*	1 to 6 or more
♀	10 to 30 or more	1 or 2	6 to 20 or more
Number of pairs of copulatory organs ..	1 or 2; modified ventral cirrus present	Only 1; ventral cirrus absent	1 to 6 or more; modified ventral cirrus present
Setigerous support of copulatory apparatus ..	2 acicula and 1 compound seta	2 acicula only; compound seta not present at any stage	2 acicula only; one compound seta present in the early stage
Sperms—From Male	Short, thick, non-motile	Slender, non-motile	Slender, long, non-motile
From Female	Non-motile, spherical, with stiff processes	Non-motile, with anterior hood	Non-motile, with broad anterior end
Number of ovarian groups ..	10 to 30 or more, in consecutive segments	1 or 2; each extending to 6 to 15 segments	6 to 20 or more; in alternate segments
Number of pairs of receptacula seminis ..	10 to 30 or more	1 or 2	6 to 20 or more
Parapodia of segment carrying receptacula seminis ..	Unmodified	Completely atrophied; dorsal cirrus and a stump supported by 2 acicula remain	Unmodified; a cirriform process developed at base

was known about their reproductive structures. Hartman (1939) clarified the systematics of the different species assigned to this genus, but his revision of the generic characters was, again, based on some of the external characters only. The detailed investigations of the morphology and anatomy of the three species, *P. gopalai*, *P. complexa* and *P. remota*, have revealed several interesting features particularly in their reproductive system; and have made possible a more specific definition of the genus. The following tabular statement gives the important characters of the four species included in the genus and indicates the salient generic features.

Undoubted morphological similarity between the species, particularly in the nature of the head, the structure of the parapodium and its setigerous support is obvious, and living as they are under more or less identical conditions, almost equally exposed to the influence of the physical factors operating on the sandy beach, this similarity is perhaps only natural. However, beneath these fundamental generic characters, important specific features are outstanding. The conspicuous caudal glands and the slightly elongated ventral cirrus of the first parapodium in *P. gopalai*; the simple semi-circular anal segment and the short ventral cirrus of the first parapodium in *P. complexa*; and, the highly elongated ventral cirrus of the first parapodium, the flask-shaped ventral cirrus of the buccal parapodium, together with the semi-circular anal segment in *P. remota*, are remarkably constant external features that easily distinguish the different species.

In the reproductive system, the formation of specialised copulatory structures for sperm transference, modification of the nephridial duct into a simple sperm-sac for the temporary storing of sperms; elaboration of wide-mouthed ciliated funnels for conducting the genital elements; the development of seminal vesicles for receiving sperms during copulation and the peculiar metamorphosis of the sperms before fertilization, are important features that are common in the three Madras species; while, a series of constant structural differences in the morphology of the different organs, distinguish the species from one another.

In the light of our present knowledge of the morphology and anatomy of the four species included in the genus, the generic characters of *Pisione* may be summarised as follows:

Pisionidæ with greatly reduced head; a pair of buccal spines; ventral cirrus of buccal parapodium rounded or flask-shaped; dorsal cirrus elongate; ventral cirrus of the first setigerous segment also elongate, and functioning as tentacular cirrus; dorsal cirrus globular as in the succeeding segments; genital papillæ in the form of suckers absent; parapodium simple

sub-biramous, with one or two simple setæ and three or four compound setæ, usually of two kinds; gonads localised to the middle and posterior segments; nephridia with solenocytes; the nephridial ducts in the male genital segments modified into sperm-sacs; one or more pairs of parapodia in the male transformed into copulatory structures which are non-retractile; paired receptacula seminis following the ovaries in the female, and sperms non-motile and undergoing metamorphosis after transference to the receptaculum seminis of the female.

Affinities of the Family Pisionidæ Levinsen

The Pisionidæ is an aberrant family of small-sized polychætes, generally of cryptic habits. Only two genera, *Pisione* Oersted and *Prægeria* Southern, were included in this family. Hartman (1939) created a new genus, *Pisionella*, and showed that Southern's genus, *Prægeria* is synonymous with *Pisione* Oersted and recognized only three species, *Pisione ærstedii* Grube, *P. remota* (Southern) and *Pisionella hancocki* Hartman, as valid. The investigations on the fauna of the sandy beach, Madras, initiated by the writer in 1938, have resulted in the addition of a remarkable new genus and species, *Pisionidens indica* Aiyar and Alikunhi, and two new species of *Pisione*, *viz.*, *P. gopalai* and *P. complexa*; besides the record of *P. remota* from the same locality.

The existing accounts of *P. ærstedii*, *P. remota* and *Pisionella hancocki* are based on the external characters of a limited number of specimens of each and as such, practically nothing is known about their internal morphology and reproductive behaviour. The affinities of the Pisionidæ were, therefore, so far considered only in the light of their known external features. Grube (1857) expressed the opinion that the Pisionidæ is related to the Phyllodocidæ and the Glyceridæ. Ehlers (1901) believed that *Pisione* is related to the Aphroditidæ, Nephthydidæ, Hesionidæ, Syllidæ and Glyceridæ. Southern (*loc. cit.*) considers the family to be closely related to the Aphroditidæ, particularly to the sub-family Sigalioninæ. According to Hartman (*loc. cit.*) their affinities are possibly with the Hesionidæ on the one hand and with the scale-bearing chætopods or more nearly, the Sigalioninæ on the other. Hartman's revised statement of the characters of Pisionidæ is also based entirely on the external features of the three species *P. ærstedii*, *P. remota* and *P. hancocki*.

Detailed accounts of the internal morphology and reproductive behaviour of *Pisionidens indica* (Aiyar and Alikunhi, *loc. cit.*), *Pisione gopalai* and *P. complexa* (Alikunhi, *loc. cit.*) are now available; and with the description of the reproductive structures of *P. remota* furnished in the present

communication, it is considered desirable to examine the affinities of the family in the light of the important internal features of the different species.

Agreeing with Grube, Aiyar and Alikunhi (*loc. cit.*) found *Pisionidens indica* more closely related to the Phyllodocidæ and the Glyceridæ than to any other family of polychætes. Subsequent studies by the writer on *Pisione gopalai* and *P. complexa* also lend further support to this view. Southern (*loc. cit.*) concluded that the palps of the Pisionidæ are homologous to the palps of the Sigalioninæ, and that the buccal segment is homologous to the segment bearing tentacular cirri in *Pholæ*. The cephalic region of *Pisionidens indica*, with the characteristic palps and tentacular cirri and the absence of buccal spines, however, shows a close similarity to the corresponding region in certain Phyllodocids like *Eteone*. The undoubted relationship between *Pisionidens* and *Pisione* has already been fully demonstrated by the structural similarities in their excretory and reproductive systems. While in *P. indica* the parapodia are uniramous, with a slender acicular bristle as the only setigerous support of the foot, in the earlier stage, atleast in the first six segments, the parapodia are temporarily provided with two acicula and four setæ which are almost identical with those in the foot of *Pisione*. As in the Phyllodocidæ, the foot in the Pisionidæ is greatly reduced and is almost uniramous (uniramous in *Pisionidens*) with the notopodium represented by a single aciculum. The resemblance of the compound seta of *P. remota*, with that of *Pholæ* which Southern points out, is rather vague; while the composite bristles from the foot of *Pisionella hancocki* figured by Hartman (*loc. cit.*) show a closer resemblance to corresponding bristles of scale-bearing chætopods. In the nature of the articulation of the terminal blade, the long-bladed compound seta in the foot of *Pisione* shows a greater resemblance to the median and inferior setæ in the foot of *Glycera*. In the possession of a protrusible pharynx, with a crown of papillæ and four jaws, the Pisionids resemble the Aphroditidæ, certain Hesionids and the Glyceridæ. In the Phyllodocidæ, however, even though the pharynx is not usually armed (except in *Phalacrophorus* (Fauvel, 1923), it is easily protrusible and muscular.

The ecological adaptations of the different forms to particular habitats of each will be largely reflected as modifications in external features; and similarities or differences in these characters should not therefore be taken to represent in full the true affinities of the concerned species. The influence of environment on features of internal morphology will be relatively limited and these characters may be reasonably considered as giving a true indication of the affinities; evidence from external characters supplementing the former. The structure and arrangement of the nephridia and genital funnels in the four species of Pisionids studied, have an important bearing

in this connection. The nephridia in all the four species are of the closed variety—the protonephridia type of Goodrich, and are provided with solenocytes. The nearest approach to the Pisionid nephridium is met with in the Phyllodocidæ, Glyceridæ and the Nephthydidæ, and such a resemblance in an important anatomical feature cannot be without significance. In the Pisionidæ the nephridia are very much simpler in structure than in the Phyllodocidæ but their general resemblance to those of *Eteone*, etc.; have already been pointed out. In *P. indica* the nephridia are so well developed in the middle segments that they are very similar to those of *Phyllodoce paretti*, eventhough there is no conspicuous branching of the nephridial swelling. The peculiar structure of the solenocytes of the Pisionid nephridium has already been described (Alikunhi, 1947). In the Aphroditidæ, on the contrary, the nephridia are of an entirely different kind, the open funnel type.

The development of conspicuous ciliated organs (Cœlomoducts) and their intimate association with the nephridia are features which the Pisionids share in common with the Phyllodocidæ, Glyceridæ and Nephthydidæ. The mode of union between the genital funnel and the nephridium is the same as in the Phyllodocidæ and Glyceridæ (Goodrich, 1900); and as in the former species the genital funnel attains communication with the nephridium only at the time of sexual maturity. In the Nephthydidæ the ciliated organs have not been known to attain communication with the nephridium; while in the glyceridæ the former are developed even in the young individuals, though, in some like *Goniada* only they attain communication with the nephridial duct.

Among other reproductive structures, the occurrence of receptacula seminis appears to be a special environmental adaptation in the Pisionidæ, even though structures of similar function have been found in the Alciopinæ (Phyllodocidæ). Spermathecæ are also developed in such different families like the Syllidæ, Hesionidæ, Spionidæ, *Saccocirrus*, etc., and should be considered as derived independently. It must, however, be mentioned that the Pisionids present a distinct advance over most families of polychætes, in the localisation of gonads, in the very restricted distribution and constancy in position of the genital funnels, receptacula seminis, sperm-sacs and copulatory organs with regard to the position of gonads, in the development of complicated copulatory organs, in the non-motile condition of the flagellate sperms and in their final peculiar transformations.

Revision of Characters of Pisionidæ Levinsen

In the light of our present knowledge of the different species included in the family Pisionidæ, the following revised statement of the family features is furnished:

Head reduced; prostomium without antennæ, and fused with the buccal segment; a pair of well developed palps; dorsal cirri of the buccal parapodia elongate and directed forwards; ventral cirri of the buccal parapodia present or absent; one or two pairs of tentacular cirri carried on distinct segments; one or two pairs of eyes; brain with elongated posterior lobes; pharynx protrusible and provided with four jaws and a crown of papillæ; parapodia uniramous or sub-biramous, with globular dorsal and ventral cirri; simple as well as compound setæ; a pair of anal cirri; body rich in epidermal glands; nerve cords situated in a groove formed by the inner edges of the ventral longitudinal muscles; deccusation of the oblique muscles beneath the nerve cord; nephridia of the closed or protonephridia type, provided with solenocytes; ciliated organs associated with them in the genital segments during the breeding season; sexes separate; gonads highly localised; genital papillæ in the form of well developed suckers present or absent; nephridial duct in the male genital segments modified into sperm-sacs; copulatory organs formed in one or more segments in the male; sperms flagellate—with one or two flagella; but non-motile; and undergoing modifications after copulation; paired receptacula seminis in the female; and fertilisation probably internal.

Summary

1. The occurrence of *Pisione remota* (Southern) in the tropical environment at Madras is recorded and the external characters of specimens from Madras compared with those from Irish waters.

2. The reproductive structures in the two sexes have been described in detail.

3. While distinct specific features are found in the detailed structure of the accessory reproductive processes, a strong resemblance to the condition in *P. gopalai* and *P. complexa* is observed.

4. The salient features of the four valid species of the genus *Pisione* have been tabulated and based on this the generic characters have been revised.

5. The affinities of the family Pisionidæ have been considered in the light of our knowledge of the anatomy of *Pisionidens indica*, *Pisione gopalai*, *P. complexa* and *P. remota*, and probable relationship to the Phyllodocidæ, the Glyceridæ and the Nephthydidæ has been indicated.

6. A revised statement of the characters of the family Pisionidæ has been given, incorporating in it our knowledge of the internal morphology of the species included in the same.

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REFERENCES

Aiyar, R. G. and Alikunhi, K. H. .. "On a new Pisionid from the sandy beach, Madras," *Rec. Ind. Mus.*, 1940, **42**, 1.

———— .. "Change of the generic name *Pisionella* Aiyar and Alikunhi, 1940, into *Pisionidens* (Polychæta)," *Curr. Sci.*, 1943, **12**, 4.

Alikunhi, K. H. .. "On a new species of *Prægeria* occurring in the sandy beach, Madras," *Proc. Ind. Acad. Sci.*, 1941, **13**, 3.

———— .. "On *Pisione complexa*, n. sp., from the sandy beach, Madras," *Proc. Nat. Inst. Sci. India*, 1947, **13**, 3.

———— .. "Genital papillæ (suckers) and sperm-sacs in the Pisionid polychæte, *Pisionidens indica* (Aiyar & Alikunhi), *Proc. 35th Ind. Sci. Congr., Patna*, 1948.

Augener, H. .. "Polychæta II. Polychæten von Neuseeland. I. Errantia, *Vid. Medd. Kjobenhavn*, 1924, 75.

David Sharp .. "Insects, Pt. II, *Cambridge Natural History*, 1918, 6.

Ehlers, E. .. *Polychæten des magellanischen und Chilensischen strandes*, Berlin, 1901.

Fauvel, P. .. *Faune de France. Polychetes Errantes*, Paris, 1923.

Goodrich, E. S. .. "On the nephridia of Polychæta, Pts. I to III, *Quart. Jour. Micr. Sci.*, 1897–1900, 40, 41, 43, n. s.

Grube, A. E. .. "Annulata Orstediana," *Vid. Medd. Kjobenhavn*, 1857.

Hartman, Olga .. "Polychæta—Families Aphroditidæ to Pisionidæ," *Allan Hancock Pacific Exped. Rept.*, 1939, **7**, no. 1.

Smith, J. E. .. "The in-fauna of the shell-gravel deposits of the eddystone grounds," *Jour. Mar. Biol. Assoc.*, 1932, **18**.

Southern, R. .. "Archiannelida and Polychæta—Clare Island Survey, *Proc. Roy. Irish Acad.*, 1914, **31 B**, pt. 47.

SOME STAGES IN THE DEVELOPMENT AND BIONOMICS OF *OCYPODA PLATYTARSIS**

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This paper gives an account of some of the stages of development of the Brachyuran *Ocypoda platytarsis*. In the literature available I have not come across a description of the developmental stages of this species. Joceyln Crane (1940) has given a description of the embryonic development of *O. gaudichaudii*. A description of the megalops of *O. albicans*, *O. gaudichaudii* and *O. occidentalis* is given by the same author. References are also given to the accounts of the zoea larvæ of *Uca pugilator*, *U. pugnax*, *U. minax* by Hyman (1920). Aikawa (1929) has described the first Zoea stage of *Macrophthalmus dilatatus*, *M. Japonicus*, *M. depressus*, *Scopimera globra* and *Ilyoplax pusillus*. Readers interested may refer to the papers mentioned for details.

After a good deal of fruitless search I was able to capure the first one, with eggs yellow and another on 26th February 1949 with the huge egg mass dark in colour. The eggs of the first specimen failed to develop. The description that follows relates to the stages obtained in the laboratory from the hatching out of the eggs of the second specimen which was taken 50 yards away from the sea, from a deep hole.

It was a robust individual, abdomen being stretched almost backward due to the mass of eggs carried (Pl. I, Fig. 1).

The dark colour of the cluster of eggs was at first thought to be due to some fungus growth. Examination under the microscope revealed the very interesting fact that the colour was due to the pigmentation of the larvæ inside the eggs (Pl. I, Fig. 2) which were ready to hatch. The egg membranes were transparent and so the pigmentation of the larvæ could be seen. Some of the eggs were transferred to a clean basin full of sea water with sea weeds at about 6 p.m. and then the crab was killed and preserved. The hatching of the eggs began on 27-2-49 and lasted till 3-3-49. Since all the eggs did not hatch at the same time it was easy to get the prezoea and zoea larvæ of

* Part of thesis submitted for M.Sc.

first stage and zoea larvæ of second stage. Zoea larvæ of second stage lived for two days and then died in contrast to the zoea larvæ of *O. gaudichaudii* (worked by J. Crane) which hatched out and died 24 hours after hatching.

Early stage.—The larva is fully formed. The eyes are strongly pigmented. On either side of the eyes there is a chromatophore subdivided into two and a number of pigment masses of varying size, rather scattered. The abdomen is strongly pigmented. There are well-developed masses of pigment on the dorsal side of the segments. Those of the first and second run together. The telson is devoid of pigment. The two pairs of maxillipeds which are conspicuous have got their bases characterised by chromatophores. The larva, about to be hatched, is conspicuous and has yolk just behind the carapace. Pigmentation is seen on the posterior middle region of the carapace just in front of the abdomen on the sides, postero-laterally on a level with the median pigmented portion.

Prezoea.—The pigmentation is same as in the previous stage. A tiny little rostrum is noticed. The dorsal and lateral spines are not developed. The abdomen is bent and seems to consist of five joints and a telson. The appendages are same as in the zoea stage (to be described) but not so well defined.

Zoea Larva (Fig. 1).—This stage is reached a day after hatching. The carapace is almost oval. There is a deep cleft through which the abdomen comes out. Lateral and dorsal spines are present in this stage. The abdomen which is curved in the prezoea stage is straightened. Pigmentation noticed in the prezoea stage is still conspicuous in this stage.

The zoea larvæ do not swim at the surface but are seen at the bottom of the basin and exhibit active movements.

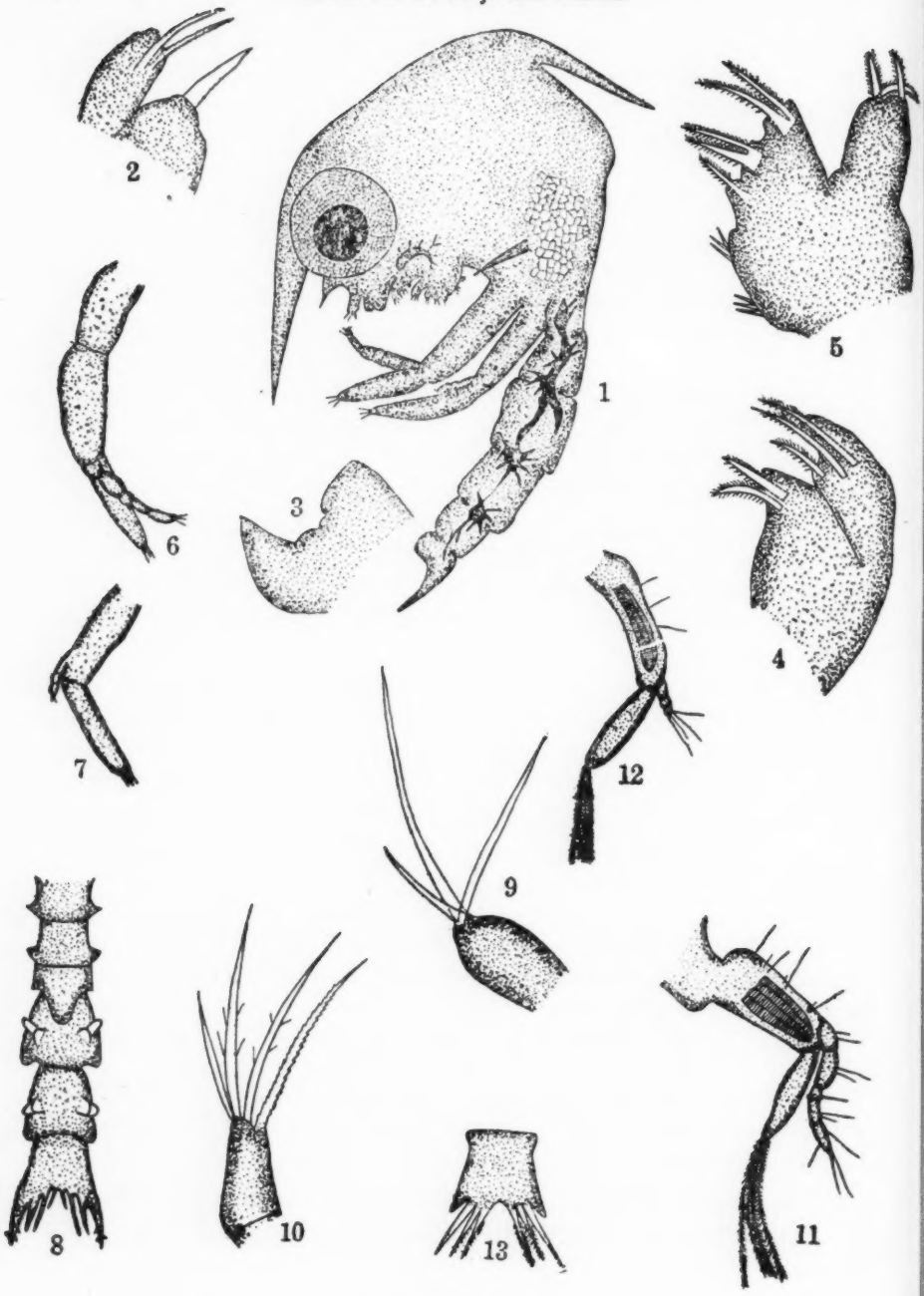
Antenna I (Fig. 2).—It is an unjointed process with a single æsthete.

Antenna II (Fig. 2) consists of a spiny process and a short exopodite with one æsthete.

Mandible (Fig. 3) is as shown in the figure.

First Maxilla (Fig. 4) is formed of two endites of which the proximal is armed with two setæ and the distal slightly curved with a single seta below and two higher up.

Second Maxilla (Fig. 5) consists of two endites one tipped with three setæ whereas the other is armed with 2–2–1 setæ, the scaphognathite with 3–3 setæ.



FIGS. 1-13

FIG. 1. Side view of zoea stage. Pigmentation in the abdomen appears to be continuous. The rostral, dorsal and lateral spines are shown, × 60.
FIG. 2. I and II antennæ, × 440.
FIG. 3. Mandible, × 440.
FIG. 4. I Maxilla of zoea stage, × 440.
FIG. 5. II Maxilla of zoea stage, × 440.
FIG. 6. I Maxillipede zoea stage, × 100.
FIG. 7. II Maxillipede of zoea stage, × 100.
FIG. 8. The abdomen (five segments and telson of zoea stage, × 100).
FIG. 9. I Antenna of advanced zoea stage, × 440.
FIG. 10. II Antenna of advanced zoea stage, × 440.
FIG. 11. I Maxillipede of advanced zoea stage, × 100.
FIG. 12. II Maxillipede of advanced zoea stage, × 100.
FIG. 13. View of the telson of the same stage from above × 100.

First Maxillipede (Fig. 6).—The protopodite is unarmed. The endopodite is five jointed and the distal joint bears three short setæ. The coxopodite also bears three short setæ.

Second Maxillipede (Fig. 7).—The protopodite is unarmed. The segmentation of the endopodite is not distinct and distally bears two setæ. The exopodite is armed with three short setæ.

The abdomen (Fig. 8) consists of five segments and a telson. Fourth segment bears a pair of knobs anteriorly directed and the fifth segment bears a pair posteriorly directed unlike the zoea larvæ of *O. gaudichaudii* where the second and third segments of the abdomen carry knobs. The telson is forked. There are three or four setæ on the forks. Between the two-pointed forks there are six setæ. The pigmentation of the abdomen same as in the previous stage.

Zoea larva—Second stage.—This stage is observed second day after hatching. The most important change is a considerable lengthening of the setæ of the maxillipede (exopodite). Pigmentation continues to be distinct.

Antenna I (Fig. 9).—It is still an unjointed process with three setæ.

Antenna II (Fig. 10)—is two jointed and the distal joint bears three setæ. The spiny process is also present.

Maxilla I.—Not very much different from that of previous stage.

Maxilla II.—The scale is bordered with many more setæ.

Maxillipede I (Fig. 11).—The protopodite is armed with 1–2–2–2 hairs. The muscles of the protopodite are easily seen in this stage. The endopodite is five jointed and is armed with 4–2–1–2–2 setæ (from distal segment onwards). The setæ of the exopodite are four in number and they are considerably longer and plumose.

Maxillipede II (Fig. 12).—The protopodite is armed with 1–1–1 hairs and at this stage the muscles of this region are conspicuous. The endopodite is three jointed with 4–0–0 setæ. The exopodite is armed with four long plumose setæ.

The abdomen consists of five segments and a telson, Fig. 13. The forks of the telson are somewhat atrophied and between the two forks there are six setæ. The central four setæ are plumose.

At this stage the larvæ perform a number of movements especially with the I and II maxillipedes. The dorsal and lateral spines are not as long as in the zoea larvæ of other forms. Even in this stage the larvæ do not swim at the surface and always rest at the bottom. There is no doubt that the swimming stage is abbreviated in *O. platytarsis*. After this stage the larvæ died in spite of my changing water very frequently.

The question whether the berried ocypoda goes to the water to liberate the eggs at the proper time must now be considered. I have not come across any case of a crab with advanced larval stages being actually encountered in the water. The literature available suggests that in one or two cases the specimens were obtained from burrows. In other cases, the information is not given. My berried specimen was obtained from a deep hole right away from the sea. The whole mass of eggs contained larvæ in advanced stages of development. The colour, commonly orange, observed by other workers as well as myself in the first specimen was very dark in the second and due to the development of extensive pigmentation of the larvæ. A comparison with zoea larvæ of other forms like *Uca* and related genera reveals the significant fact that in *O. platytarsis* the spines are comparatively shorter than in other cases. This, to my mind, suggests that the genus *Ocypoda* generally and in *O. platytarsis* particularly the swimming period is greatly abbreviated as it would be natural in a form which is becoming progressively terrestrial. This is in close analogy with a number of polychætes in which the ciliated rings and primary parapodia show considerable reduction in forms which have become very sedentary and taken to almost terrestrial system. Krishnan (1936) has shown that in *Diopatra* a swimming stage has been completely given up and this modification has been further exemplified by Ranganathan (unpublished) in the development of *Lycastis* in which the eggs undergo their development in minute cocoons attached to the cirri. I am therefore inclined to believe that the zoea stage is very transitory. The suggestion is hazarded that those zoea larvæ hatch out at the bottom of their burrows where enough moisture is present.

Bionomics

Ocypoda platytarsis like any other *ocypoda* is a keen-eyed, swift runner darting away with lightning speed during day time when a man is even 30 feet from the crab. During dark nights though the eyes are erect, the crab is not able to see even when approached close to it. When a torch is flashed on it, it tries to run away. The observation of Cowles regarding *O. arenaria* (1908) is true for this form also. He remarks: " Ocypodes probably do not have vision such as that of the human eye nor do they see the colour and the finer characters of the surface of the object but they undoubtedly see its outlines and probably some of the more evident irregularities of the surface made evident by differences in lighting."

Colouration.—The colouration of the very young crabs is in perfect harmony with the surroundings. The colour pattern for the mature male and female is given below.

Male.—Carapace—Brown mottled chelipeds and walking legs light brown. Eyes—black.

Female.—Carapace, terminal style appendages bright yellow. Visual region—black.

Sometimes individuals with carapace and legs bright yellow have been observed. It has been noted that the yellow colour is more common in males than in females. Whether this is a sort of sex colour could not be determined as the phenomenon of courting has not attained in this form that degree of development which is so characteristic in the species of (Gelasimus) uca.

Growth of the Carapace.—Carapace measurements of *O. platytarsis* (male and female) collected at Vizag and Waltair gave interesting results. The carapace measurements were taken from the tip of the rostrum to the median hind border of the carapace and from one end of the anterio-lateral border to the other at the broadest part.

Breadth of carapace of a female crab is generally more than that of a male crab for the same length of carapace. However, the breadths are nearly the same within the range of 16 mm. to 25 mm. of carapace lengths.

The largest female caught measured 38 mm. long and 48·5 mm. broad.

The largest male showed the following dimensions:—Length 41 mm. and bradth 50 mm. The crab appears to mature when the length is around 24 to 26 mm. But this point can be settled only when more data are available.

The terminal style grows only when the crab is around 12 mm. in length. This is midway between what is observed in the case of *O. gaudichaudii* (7 mm.)

and *O. ceratophthalma* (20 mm.) (Crane, 1941). For a length of about 17 mm. the styles are almost of the same size in both the sexes. From 17 to 23 mm. length of carapace, the length of the style in the male is more than that of the female. From 23–32 mm. the females have longer styles. From 32–41 mm. carapace length, there is not much difference between the style lengths in both the sexes.

Construction of burrows.—The method of building burrows is similar to that of any other ocypode. The sand is carried by the palm of the minor cheliped and also by the I and II walking legs of the same side. The material to be rejected is sometimes flicked with considerable force away from the burrow or sometimes just dumped near the burrow. The crab usually rests after this act for a minute or so and then disappears down the burrow. After the lapse of a few minutes it reappears with a fresh load of excavated sand.

Near each burrow a pyramid of wet sand could be seen during low tide since repairing of the burrows goes on vigorously at this time.

Feeding.– High water line is the favourite ground of feeding. This has been found to be so by other workers also. As soon as the wave recedes, the crab goes a bit away from its burrow to feed. It is during high tide that young of *Hippa*, fish scales, broken parts of internal skeleton of sepia and other debris are brought in large quantities and deposited on the shore by the receding wave. Pearse (1929) remarks that the Ocypode is an omnivorous scavanger and beach-comber. It is too true of *O. platytarsis.* It feeds on fish remains, on other marine crabs, *Hippa,* weeds and anything like bread pieces, cocoanut husk and dung, etc., cast up by the waves.

In feeding both chelipeds are used simultaneously to hold the food or the major and the minor chelipeds may be used alternately.

Cannibalism is also seen among *Ocypoda platytarsis.* Two medium sized crabs and three small crabs were placed in a beaker at about 4 p.m. in the evening. The next day all the three small crabs were found torn to pieces. The soft parts had been eaten and the hard portions alone left behind.

Another curious phenomenon of these crabs is, any article of food matter—if it is small enough—is taken inside the burrow and deposited at the very bottom of the hole. Pieces too big to be taken into the burrows are left at the entrance conveniently near for the crab to come out and feed now and then. Once during low tide *O. platytarsis* was observed to be feeding on a dead *Matuta victor.* Evidently the crab must have dragged it and placed it adjacent to its burrow.

Behaviour of the Crabs to Water.—O. platytarsis likes now and then to immerse itself in water. Then several of them are found in the inter-wave zone, so to speak hugging the sand and flattening themselves when the waves pass over and subsequently retreat.

During high tide, the beach is alive with innumerable crabs of all sizes. This habit is peculiar to *O. platytarsis*. No other worker on Ocopodes has mentioned such a habit. Some rest at the entrance of their burrows with the minor cheliped side inside and the major chela side away from the burrow. The large nipper is bent whereas the perieopods are stretched out. The crabs, with their eyes erect, give the idea of being intelligent, alert and vigilant. Some of them go a few paces away from their burrows and come back to their holes with extraordinary speed after a few seconds. In no case was the crab observed to be away from the burrow for more than two minutes during high tide. What is most striking is, Ocypodes do not plug their holes during high tide. They are on the beach so to speak playing actually hide and seek with the waves. The wave goes over the burrow and closes it. After a few minutes the crab emerges out of its burrow throwing out the wet sand, rests at the entrance of the burrow darting away with the receding wave in search of food. It returns to the burrow each time before the next wave comes in. By instinct the Ocypode seems to know when the wave will cover its burrow. Not one specimen was observed to be far away from the burrow or caught in the wave during high tide. Though *O. platytarsis* goes to the water edge for respiratory purposes, it cannot live for long in sea water. In fresh water they die much earlier.

Autotomy is exhibited by this crab like any other crab. The limb breaks at the fracture plane. Mites are invariably found in the branchial chambers.

Daily schedule.—High tide—*One hour prior to high tide*—number of small crabs and medium-sized crabs are seen on the beach, busy feeding and running about. This is contrary to the observations of J. Crane (1941) on *Ocypode gaudichaudii.*

Half an hour before high tide.—Big mature crabs emerge out of their burrows.

High tide.—Beach alive with crabs of all sizes playing hide and seek with the waves and also feeding.

2 *Hours after high tide.*—Crabs repair their burrows and rest at the entrance. Feeding occasionally goes on.

6 *Hours after high tide.*—Big crabs not seen. Small crabs and medium-sized crabs repair their burrows lazily and feed occasionally.

L ow tide.—Small crabs are seen running about. Mature crabs are conspicuously absent.

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BIBLIOGRAPHY

1. Aikawa, H. .. "On larval forms of some Brachyura," *Rec. Oceanogr, Wks. Jap.*, 1929, **2**, 17–55.

2. Cowles, R. P. .. "Habits reactions in *Ocypode arenaria*." *Papers from the Tartugas Laboratory of the Carnegie. Inst. Washington*, 1908, **IIk.**, 3–39.

3. Crane, J. .. "Eastern Pacific Expeditions of the New York Zoological Society, XVIII. On the post embryonic development of Brachyuran crabs of the genus Ocypode". *Zoologica*, 1940, **25**, 65–82.

3*a*. ———— .. "Eastern Pacific Expeditions of the New York Zoological Society, XXVI. Crabs of the genus *Uca* from the West Coast of Central America," *ibid.*, 1941, **26**, 145–208.

4. ———— .. "Eastern Pacific Expedition of the New York Zoological Society, XXIX. On the Growth and Ecology of the Brachyuran crabs of the genus Ocypode," *ibid.*, 1941, **26**, 297–310.

5. Hyman, O. W. .. "On the development of Gelasimus after hatching," *Journ. Morph.*, 1920, **23**, 485–524.

6. Krishnan, G. .. "The development of *Diopatara variabilis* (Southern)," *Zeitschrift fiir Wissenschaptliche Zoologie*, 1936, **47**, 513–28. Akademische Vordags gesellschapt n. b. h. in Leipzig.

7. Pearse, A. S. .. "Observations on certain Littoral and Terrestrial animals at Tortugas Florida with special reference to migrations from marine to terrestrial habitats," *Carnegie Inst. of Washington Publications*, No. 391.

8. Ranganadhan, M. .. Development of *Lycastis indica* (Unpublished).

EXPLANATION OF PLATES

FIG. 1. Ventral view of *Ocypoda platytarsis* (female) with eggs in advanced stages of development.

FIG. 2. Cluster of eggs in advanced stage of development.

FIG. 3. Enlarged view of the same.

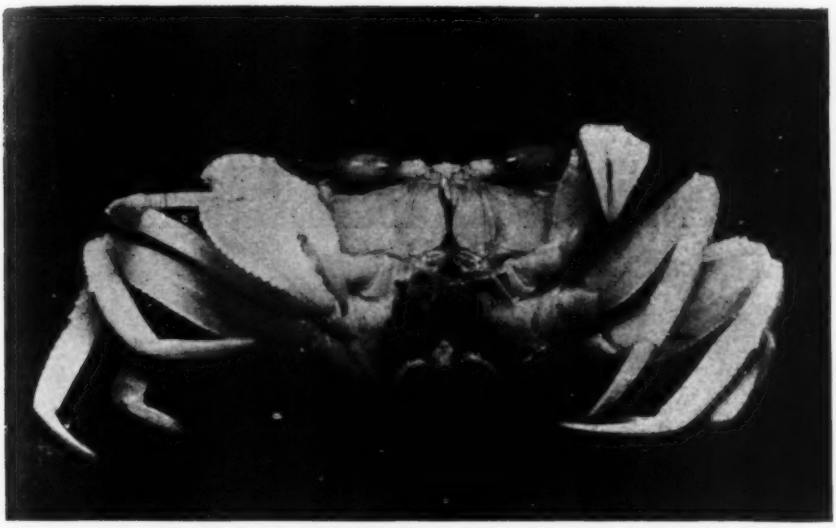
FIG. 4. Prezoea.

FIG. 5. Zoea larvæ.

FIG. 6. Ocypoda crab emerging from burrow and another with dead shell ready to fight.

FIG. 7. Winner (with shell) occupying burrow—the vanquished running away.

N.B.—Figures have been reduced to half the magnifications given.



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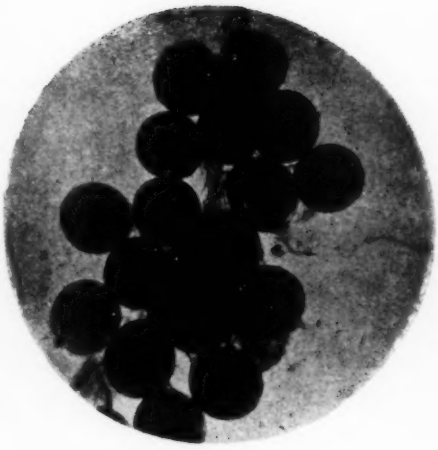

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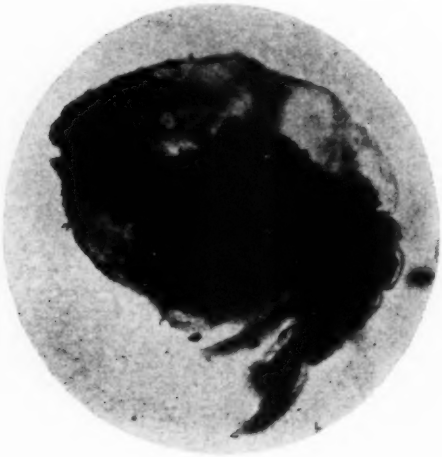

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THE STRATIGRAPHICAL VALUE OF MISCELLANEA AND PELLATISPIRA IN INDIA, PAKISTAN AND BURMA*

(With two figures)

BY Y. NAGAPPA

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Received April 15, 1950

(Communicated by Prof. L. Rama Rao)

Abstract.—In the Eocene of Pakistan, India and Burma, two genera—Miscellanea and Pellatispira—have shown a remarkably stable and limited vertical range in the several areas in which representatives of these genera are found. Miscellanea, which is represented by *M. miscella* (d'Archiac and Haime), *M. stampi* (Davies), and *M. meandrina* (Carter), is confined to the Ranikot (Palæocene age) in all the areas examined so far, while Pellatispira, which is represented by *P. cf. orbitoidea* (Provale), *P. cf. inflata* Umbgrove, *P. cf. madaraszi* Von Hantken, *P. cf. glabra* Umbgrove, is found only in the very high Eocene (Upper Eocene) beds. Because of their very restricted vertical range these two genera are of considerable stratigraphic value.

I. INTRODUCTION

THE role of foraminifera in geological history has assumed considerable importance in recent years; and foraminifera being mostly marine have proved exceptionally useful in stratigraphical palæontology. A form to be ideally suited for stratigraphical correlation should have a limited vertical range and a very wide geographical distribution and should be independent of facies. Such an ideal form is almost impossible to find in nature where faunal communities being largely dependent on ecological factors are influenced by changes in facies. So we are often faced with the problem of dealing with faunas of extreme diversity even within the same region, and in rocks presumably of the same age.

2. In the Palæocene and Upper Eocene of India, Pakistan and Burma there are two genera, Miscellanea and Pellatispira, which are known from many widely separated localities and which are probably of particular value in stratigraphic correlation.

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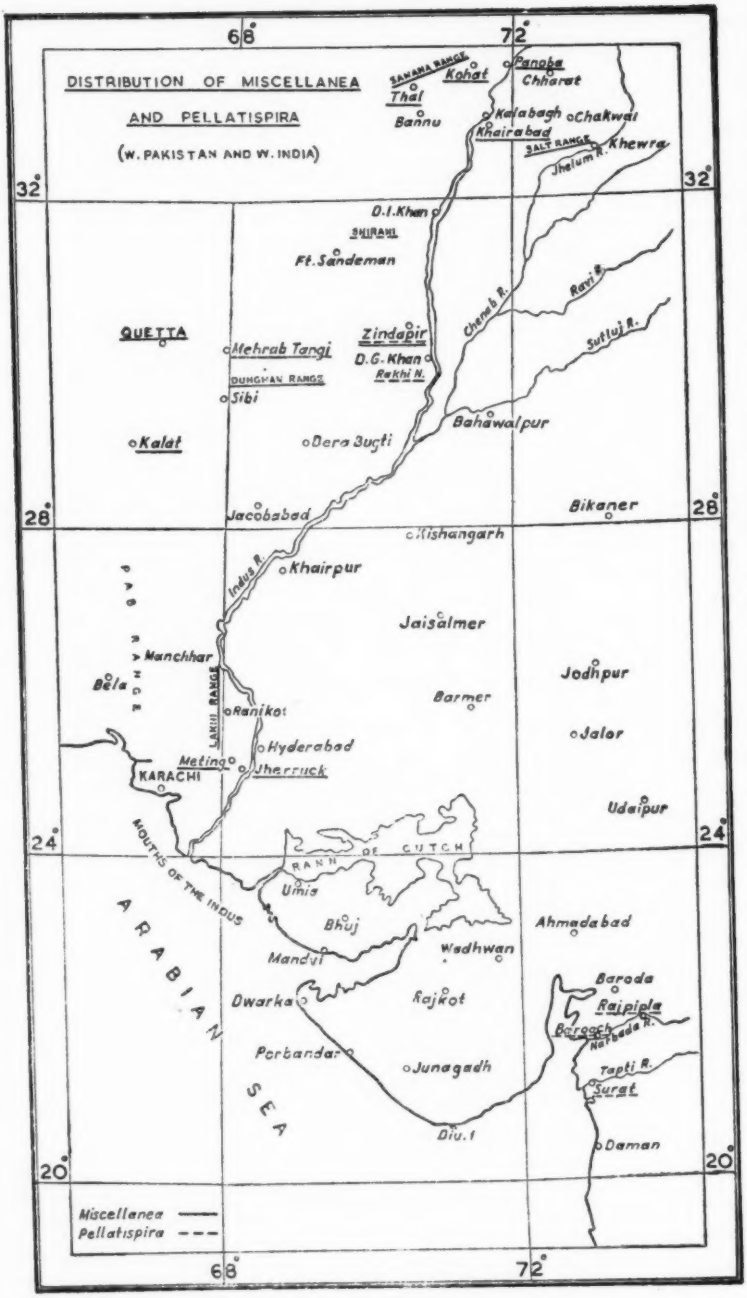
* Paper read at the 37th Indian Science Congress (Geology Section), Poona, January 1950. Published with the kind permission of The Burmah Oil Co. (India Concessions) Ltd.


DISTRIBUTION OF MISCELLANEA
AND PELLATISPIRA
(W. PAKISTAN AND W. INDIA)
68°
72°
32°
28°
24°
20°
SAWANA RANGE
Kohat
Thal
Bannu
Panoba
Chharat
Kalabagh
Chakwal
Khairabad
SALT RANGE
Khewra
Jhelum R.
D.I.Khan
SHIRANI
Ft. Sandeman
Chenab R.
Ravi R.
Sutluj R.
QUETTA
Mehrab Tangi
Zindapir
D.G. Khan
Rekhi H.
DUNGHAN RANGE
Sibi
Kalat
Dera Bugti
Bahawalpur
Jacobabad
Bikaner
Kishangarh
Khairpur
Indus R.
PAB RANGE
Jaisalmer
Manchhar
Bela
LAKHI RANGE
Ranikot
Barmer
Jodhpur
Hyderabad
Jalor
Meting
Jherruck
KARACHI
Udaipur
MOUTHS OF THE INDUS
RANN OF CUTCH
Umia
Bhuj
Ahmadabad
Mandvi
Wadhwan
ARABIAN SEA
Baroda
Rajpipla
Dwarka
Rajkot
Broach
Narbada R.
Porbandar
Junagadh
Tapti R.
Surat
Diu.I
Daman
Miscellanea
Pellatispira

II. Distribution of Miscellanea

3. Outside the India, Pakistan and Burma sphere, Miscellanea has been recorded from the Palæocene rocks of Madagascar, Syria, Basses-Pyrennees[20]* and West Indies (Trinidad, Haiti[27] and Jamaica[24]). In the areas now under consideration this genus is represented by *Miscellanea miscella* (d'Archiac and Haime), *M. stampi* (Davies) and *M. meandrina* (Carter) which are recorded in abundance from all the typical Ranikot rocks particularly wherever the development is calcareous. In Sind, the type area for the Ranikot, Miscellanea is recorded from the Lakhi Range and from the neighbourhood of Jherruck.[17, 18, 28] In Baluchistan there are isolated occurrences of rocks containing Miscellanea, *e.g.*, in Las Bela near the southern end of the Pab Range, in the neighbourhood of Quetta, in the Mehrab Tangi, in the Dunghan Mountain and in the 'Valley of Kelat'[1] (Kalat).† The genus in the Sind-Baluchistan areas is represented by *Miscellanea miscella*, *M. stampi* and *M. meandrina*. Further north, occurrences of Miscellanea are recorded from the basal Eocene succession along the foothills of the Sulaiman Range (Zinda Pir). Here the genus is represented mostly by the megalospheric form *M. miscella*. There seems to be no further record of beds containing Miscellanea until we reach the latitude of Kohat (about 33° 30′ N.). Here, *Miscellanea miscella*, the megalospheric form, with an occasional occurrence of *M. stampi* is found abundantly in the hard limestones forming the core of the Panoba dome, in the Samana Range,[7] in Thal[6] and in the Chharat area. In the Panoba dome, however, *M. meandrina* is also present.

4. To the east of this area in the Salt Range flood occurrences of *M. miscella* are recorded from the Khairabad Limestone and the Pail Limestone[8] and occasionally in the underlying Dhak Pass Beds. *M. meandrina* has, however, been found only in the Khairabad Limestone near Khairabad.

5. Little is known about the Ranikot (Palæocene) development in other areas but in the Kampa Dzong area of Tibet (28° 17′ : 88° 34′) beds containing *M. miscella* again occur and have been placed in the Ranikot.[8, 10] The associated fauna is in keeping with that of W. Pakistan.

6. There are other occurrences of the genus from parts of Assam and Burma which have not previously been recorded. The occurrences in Assam are all from the lower parts of the Sylhet Limestone where again the associated fossils are typical Ranikot forms. The Burma

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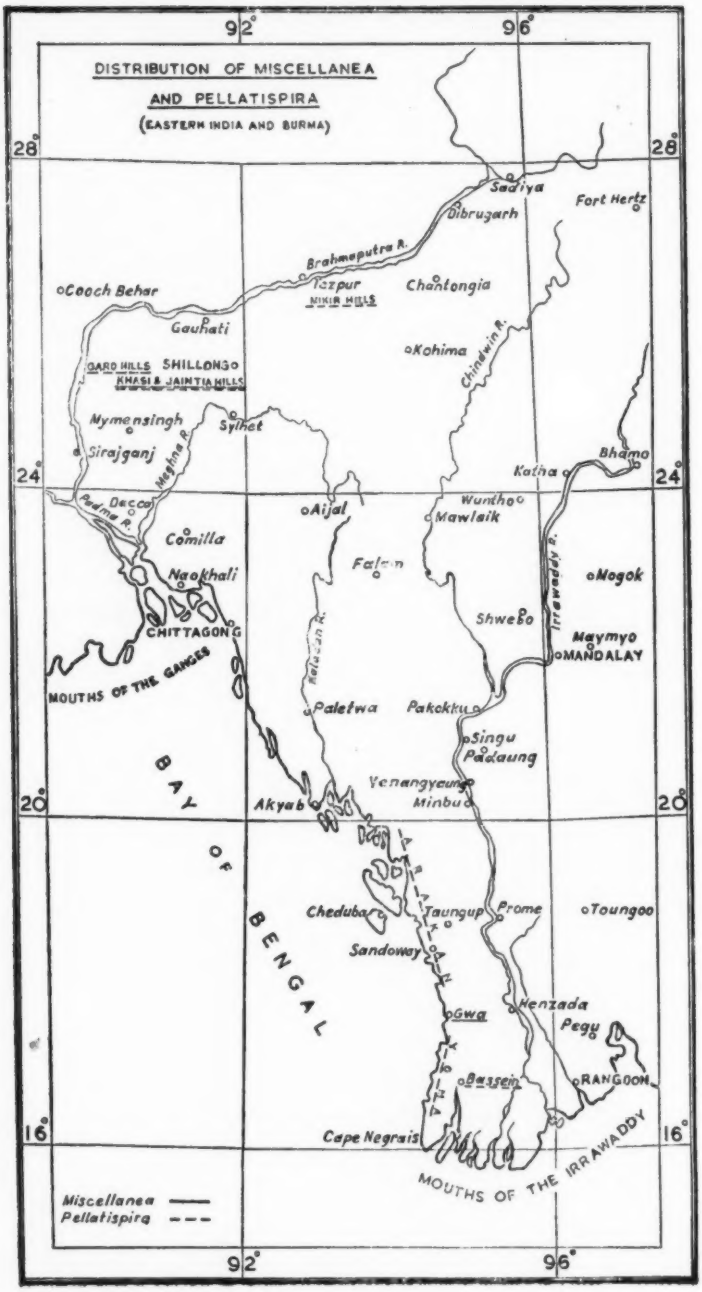
* Numbers indicate references in Bibliography.

† 29° 0′ : 66° 48′—34 K/12.


DISTRIBUTION OF MISCELLANEA
AND PELLATISPIRA
(EASTERN INDIA AND BURMA)
92°
96°
28°
24°
20°
16°
Sadiya
Dibrugarh
Fort Hertz
Brahmaputra R.
Cooch Behar
Tezpur
Chantongia
NIKIR HILLS
Gauhati
Kohima
Chindwin R.
GARO HILLS
SHILLONGO
KHASI & JAINTIA HILLS
Mymensingh
Sylhet
Meghna R.
Sirajganj
Katha
Bhamo
Padma R.
Aijal
Wunthoo
Mawlaik
Comilla
Falam
Irrawaddy R.
Mogok
Naokhali
Shwebo
Maymyo
CHITTAGONG
MANDALAY
MOUTHS OF THE GANGES
Kaladan R.
Paletwa
Pakokku
Singu
Padaung
BAY OF BENGAL
Yenangyaung
Akyab
Minbu
Cheduba
Taungup
Prome
Toungoo
Sandoway
Henzada
Gwa
Pegu
Bassein
RANGOON
Cape Negrais
MOUTHS OF THE IRRAWADDY
Miscellanea
Pellatispira

occurrences are in scattered outcrops in the western foothills of the Arakan Yoma between Gwa and Ngashandaung. In this latter locality the stratigraphical succession is not clear but there seems little doubt that they too may be referred to the Ranikot (Palæocene).

III. Distribution of Pellatispira

7. The genus Pellatispira is well known outside the India-Pakistan-Burma sphere from several widely separated areas: Europe (Italy, Sicily and Scaglia in the Apennines),[15, 25] Western Australia[13] and also from the Pacific (Tonga Islands). In every case the beds containing Pellatispira have been regarded as Upper Eocene. In Indonesia several species of Pellatispira have been recorded[25, 26] from Tertiary '*a*' and '*b*' (M. & U. Eocene). In the India, Pakistan, and Burma sphere, S. R. N. Rao (1941) has recorded this genus from the upper limestones of Surat and Broach* and on this strength he has correlated this horizon with the Upper Eocene. There are several other occurrences not previously recorded brief references to which are made below.

8. In W. Pakistan, as far as is known at present, Pellatispira is found in three localities along the eastern foothills of the Sulaiman Range namely, Rakhi Nala, Zinda Pir‖ and Shirani and occurs in the uppermost 60 ft. or so of the Upper Chocolate Clays.

9. In Western India, Pellatispira is known from Rajpipla.† So far Pellatispira has not been reported from Sind, Baluchistan and Cutch areas although Eocene rocks are well developed there.

10. In eastern India rocks containing Pellatispira are well developed in the Kopili beds[11] of the Garo, Khasi, Jaintia and Mikir Hills of Assam and rarely in the Disangs.‡ In the Kopili beds, Pellatispira is very common but in most sections the occurrences are confined to the lower part only. The higher horizons in the Kopilis are generally unfossiliferous since there is a facies change from the highly calcareous and argillaceous development in the lower part to one of arenaceous-argillaceous alternations in the upper part. Occasionally one of the species of Pellatispira§ occurs in the upper beds of the underlying Sylhet Limestone.

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* The following have been recorded by Rao:
P. madaraszi Von Hantken var. *indica* Rao.
P. inflata Umbgrove var. *minor* Rao.
P. orbitoidea (Provale).

‖ Here the genus is represented by *P. cf. madaraszi* Von Hantken.

† 73° 33′ : 21° 47′—46 G/9.

‡ Chantongia (94° 41′ : 26° 33′—83 J/10).

§ *P. cf. orbitoidea* (Provale).

11. In Burma, Pellatispira is very common in the upper part of the Pondaungs and the overlying Yaw Stage.* The occurrences are along the eastern foothills of the Arakan Yoma extending from Bassein Dist. (85 L. 16° 25′ : 94° 52′) in the south to about latitude 22° in the north; further north the facies becomes arenaceous and loses its typically marine character.

12. In all these localities the Pellatispira-bearing beds contain amongst other fossils reticulate Nummulites, large Discocyclines,† Gypsina‡ and Heterostegina and can be regarded as of Upper Eocene age.

IV. Summary

13. A rèsumè of the occurrences of Miscellanea in India, Pakistan and Burma has shown that the genus which is represented by three species, is confined to beds of Ranikot (Palæocene) age and seems to fully justify Pfender and Davies's claim that it is indeed an index for the Palæocene. From the records of the occurrences of Pellatispira in India, Pakistan and Burma it can be stated that this genus is confined to the highest beds of the Eocene in the whole region. The Pellatispira-bearing beds outside this region have been regarded as of Upper Eocene age and it seems reasonable to assume that the horizon of the Pellatispira-bearing beds in the above region is also of Upper Eocene age.

14. Both these genera thus appear to have a limited stratigraphical range within the Eocene and will no doubt prove very useful to the stratigrapher.

V. Acknowledgement

15. The writer is indebted to Messrs. W. B. Metre and A. J. Philipson for helpful suggestions in the preparation of this paper. The information on the distribution of the two genera Miscellanea and Pellatispira is taken both from published literature and from the records of new occurrences discovered by the writer and his colleagues while carrying out geological investigations for the Burmah Oil Company in this region. The writer also wishes to take this opportunity of thanking Prof. L. Rama Rao for information on the occurrence of basal Tertiary rocks in the Pondicherry area and Prof. S. R. Narayana Rao for information on some of the outside occurrences of Miscellanea and Pellatispira.

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* *P. cf. inflata* Umbgrove, *P. cf. orbitoidea* (Provale), *P. cf. madaraszi* Von Hantken and *P. cf. glabra* Umbgrove are the common species.

† *D. cf. omphalus.*

‡ Occurs in quantity in the Yaw Stage of Burma.

BIBLIOGRAPHY

1. Carter, H. J. .. "Further Observations on the structure of Foraminifera," *Ann. Mag. Nat. Hist.*, 1861, Ser. 3, **8.**
2. Cotter, G. de P. .. "Pegu-Eocene Succession in Minbu," *Rec. Geol. Surv. Ind.*, 1912, **41,** 235.
3. ———— .. "Nummulites as Zone Fossils," *ibid.*, 1914, **44,** 53.
4. ———— .. "Age of the so-called Danian Fauna from Tibet," *ibid.*, 1926, **59.**
5. Davies, A. M. .. *Tertiary Faunas*, 1934, 1935, Vols. 1 and 2.
6. Davies, L. M. .. "Ranikot Beds of Thal," *Q. J. G. S.*, 1927, **83.**
7. ———— .. "Fossil Fauna of the Samana Range," *Pal. Ind.*, N.S., 1930. **15** (1 and 6).
8. ———— .. "The Eocene of the Punjab Salt Range," *ibid.*, N.S., 1937. **24** (1), Part 2.
9. Douville, H. .. "Le Crètacè et l'Eocène du Tibet Central," *ibid.*, N.S., 1916, **5** (3).
10. Ellis and Massina .. *Catalogue of Foraminifera.*
11. Evans, P. .. "Tertiary Succession in Assam," *Trans. Min. Geol. Inst. India*, 1932, **27** (3).
12. Furon Raymond .. *Geologie de L'Inde Orientale Francaise*, Pub. Bureau d'Etudes Geol. et Min. Coloniales, 1941, No. 17.
13. Glaessner, M. F. .. "Problems of Stratigraphic Correlation in the Indo-Pacific region," *Proc. Roy. Soc. Vic.*, N.S. 1943, **55,** Part 1.
14. Hoffmeister, J. E. .. "Geology of Eua, Tonga," *Bernice P. Bishop Mus. Bull. Honolulu, Hawaii*, 1932, No. 96.
15. Narayana Rao, S. R. .. "Tertiary Sequence near Surat and Broach," *Jour. Mys. Uni.*, Sec. B, 1941, **2** (2).
16. Nuttall, W. L. F. .. "The Laki Series of Sind and Baluchistan," *Q. J. G. S.*, 1925, **81** (3).
17. ———— .. "The Larger Foraminifera of U. Ranikot, Sind," *Geol. Mag.* 1926, **63.**
18. ———— .. "The Stratigraphy of the U. Ranikot of Sind," *Rec. Geol. Surv. Ind.*, 1931, **65** (2).
19. ——— and Brighton, A. G. "Larger Foraminifera from the Tertiary of Somaliland," *Geol. Mag.*, 1931, **68,** 60.
20. Pfender, J. .. *Bull. Soc. Geol. Fr.*, Ser. 5, 1936, **6,** p. 308.
21. Rama Rao, L. .. "On the Occurrence of an Eocene bed in the Pondicherry Cretaceous area, S. India," *Current Science*, 1939, **8,** No. 4.
22. ———— .. "On *Nummulites cf. thalicus* Davies from the Eocene bed in the Pondicherry area, S. India," *ibid.*, 1940, **9.**
23. ———— .. "Recent Advances in our knowledge of the Upper Cretaceous and Lower Eocene beds of India with special reference to the Cretaceous-Eocene boundary," *Presidential Address, Geol. Sec., 27th Ind. Sci. Congress*, 1940.

24. Shimer, H. W. and Shrock, R. R. *Index Fossils of North America*, 1947.

25. Umbgrove, J. H. F. .. "Het genus Pellatispira in het Indo-Pacifische gebied," *Weten. Med.*, 1928, No. 10.

26. ———— .. "Feestbudel Prof. K. Martin," *Leidische geol. Med.*, 1931, **5**, p. 53.

27. Vaughan, T. W. .. "American Palæocene and Eocene larger Foraminifera," *Geol. Soc. Amr. Mem.*, 1945, **9**, p. 23.

28. Vredenburg, E. .. "Note on the Stratigraphy of the Ranikot Series," *Pal. Ind.*, N.S. 1909, **3**, (1).

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SOIL CONDITIONS AND ROOT DISEASES

Part II. *Fusarium udum* Disease of Red Gram [*Cajanus cajan* (Linn.) Millsp.]*

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University Botany Laboratory, Madras

Received April 24, 1950
(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

It has become increasingly evident during recent years that much stress is being laid by Plant Pathologists throughout the world, especially in the U.S.A., Canada and England, for a better understanding of soil conditions and the development of soil-borne diseases of crops and plants. Indeed, the emphasis has shifted from non-living factors of the soil such as temperature, water-holding capacity, aeration and reaction to the influence exerted by the associated micro-flora on the growth and persistence of the pathogen.

Recently, however, many workers have demonstrated successfully the possibilities of biological control of soil-borne diseases with application of organic and inorganic nutrients to the soil, on the basis of the concept of micro-biological antagonism as a potential force for disease control [Sanford (1926); Millard and Taylor (1927); King, *et al.* (1934); Mitchell, *et al.* (1941)]. Shear and Wingard (1944) have called attention to experimental evidence that brings out possible relationships between certain unbalanced conditions in the mineral nutrition of a host plant and the severity of attack by certain vascular pathogens. Noteworthy studies on this aspect are by Smith and Walker (1941), Young (1938) and Pryor (1940). The majority of these investigations has been confined mainly to a study of the major elements. Comparatively very little work has been done with regard to micro-element and its nutritional bearing on host-parasite relationship though considerable improvement has been found (Millikan, 1938; 1942) on application of zinc sulphate in the growth of wheat crops suffering from the combined attack of a number of root-infecting fungi.

In a problem of this type where an intimate relationship exists between host and pathogen, independent studies should be made of (1) the fungal response to a substratum containing micro-elements, (2) the reaction of the

* Part of a thesis approved for the Degree of Doctor of Philosophy of the University of Madras.

plant to the minor element and (3) the response of the plant in the combined presence of the pathogen and minor element.

Yogeswari (1948) working on the trace element nutrition of the wilt fungi—*F. vasinfectum*, *F. moniliforme* and *F. udum*—from the pure culture aspect, demonstrated that all the three fungi responded well to various concentrations of zinc, boron and manganese. Optimum levels were low and differed somewhat with the three strains, although higher concentrations were not toxic. It is therefore but natural to expect that further work along these lines would have a great bearing on the general understanding of host-parasite relationships in plant wilts.

The most important factor in biological control on which has been based the work of many recent investigations—Reinking and Manns (Reinking, 1934, 1935; Reinking and Manns, 1933; 1934); Garrett (1938); Sadasivan (1939); Walker (1941)—is that there is possibly more of inhibition of the saprophytic phases of root-infecting fungi than suppression of their parasitic activity (Waksman, 1917). Indeed, it has been shown (Subramanian, 1946) that *Fusarium vasinfectum*, the cotton-wilt pathogen is highly sensitive to microbial antagonism and does not persist for long in soils due to the operation of this factor. Considerable work has been done in the United States, Egypt and India on the problem of cotton wilt. Red gram (pigeon-pea) wilt has engaged the attention of the Indian Agricultural Research Institute as early as 1907 and investigations along several lines have shown that wilt is more prevalent in clayey soils favoured by high soil moisture (McRae and Shaw, 1933) and low temperature conditions (Mitra, 1934), the incidence increasing on manuring with superphosphate and cattle manure, green manure by itself producing a beneficial effect (McRae and Shaw, 1933). According to a recent report of the Imperial Mycologist, (Padwick, 1939-40), however, application of farmyard manure hastens the disappearance of the fungus and it has been concluded after several years of manurial and rotation trials that the best possible method of control is only by breeding wilt-resistant strains.

Controversial reports prevail as to the taxonomy and nomenclature of the causal organism. Butler (1926) revised his previous opinion (1910) of considering the pigeon-pea isolate as *Fusarium udum* and decided that it was a synonym of *F. vasinfectum*, the cotton pathogen. Wollenweber (1938), however, disagreed with Butler's decision by bringing forth a number of distinguishing characters between *F. udum* and *F. vasinfectum*. The pigeon-pea parasite has been referred to as *F. vasinfectum* by numerous workers in India—McRae and Shaw (1933); Plymen (1933); Mitra (1934); Uppal

and Kulkarni (1937); Mundkur (1935); Bose (1939)—probably because of the explanation offered by Butler (1926). Recently, however, Padwick (1940 *b*) after a very critical study of the whole problem has concluded that *F. udum* is not synonymous with *F. vasinfectum* and has therefore proposed to call the wilt organism of *Cajanus cajan*, "*Fusarium udum* Butl. var. *Cajani*". However, it has been recorded that more than one species of *Fusarium* is involved in the disease—Wollenweber (1938), Padwick (1937-38)—and it is therefore interesting to note that during the course of this work, a few distinctly varying isolates of *Fusarium* have been obtained in association with wilted red-gram plants or pigeon-pea wilt-sick soil.

Materials and Methods

The general laboratory technique followed was as proposed by Rawlins (1933), for preparation of media, inoculation, maintenance of cultures, etc. Some of the special methods used for the studies are detailed below. However, it may be mentioned that any technique over and above those given below will be referred to under experimental results.

1. *Isolation of the Pathogen.*—Several isolates were obtained during the course of the investigation from diseased *Cajanus* material, from seeds which were destroyed by pre-emergence wilt when sown in wilt-sick soil and from host stubble buried in infested soil for colonization experiments.

The surface sterilizing agents used for infected plant or seed material was either 1/1,000 mercuric chloride solution or 1/14 calcium hypochlorite solution following the techniques suggested by Walker (1941).

The infected material (stubble or seed) was placed after surface sterilization on 2 per cent. potato dextrose agar acidified with lactic acid (3 ml. to 1,000 ml. of medium) and poured in Petri dishes. The *Fusarium* appearing was subcultured on potato dextrose agar in test tubes.

2. *Cultural Studies :* (Fusarium *strains*)

The standard method of recording cultural characters as suggested by Padwick (1940 *b*) for his cultural studies on "The Genus *Fusarium*" was followed.

3. *Pathogenicity Experiments :*

Fungi tried: Fusarium udum and other strains isolated in connection with pigeon-pea wilt.

Soil used.—Air dried and sieved garden 'compost' soil prepared by mixing stable manure, sand and red earth in the proportion of 2 : 2 : 1.

Soil containers.—Porcelain glazed pots (size 6·5″ × 3·5″) were used mostly, as these were found to be more suitable in maintaining sterile conditions as also the required moisture level than earthenware pots. These were autoclaved at 20 lb. pressure for 30 minutes for the unsterilized soil trials. For the sterilized series, pots filled with moistened soil were autoclaved at 20 lb. pressure for 2 hours.

Cajanus strain used.—Seeds collected from a strain of red gram plants grown in the laboratory garden plot (Susceptible variety No. 1723 of the Department of Agriculture, Madras) were surface sterilized in 1/1,000 $HgCl_2$ solution for 2 minutes and then washed in several changes of sterile water before being sown in soil, in depressions of about $\frac{1}{2}$″ to $\frac{3}{4}$″ depth.

Conditions of incubation.—All pots were incubated in the laboratory glass-house, the temperature of which varied from 29-31° C., during the course of the trials.

Soil moisture.—Determination of soil moisture was made by the Keen-Raczkowski method (Keen and Raczkowski, 1921).

Methods of inoculation.—Various methods of inoculation were tried and the oat-soil inoculum (Padwick, 1940 *a*) was found to be most useful and was, therefore, used throughout the pathogenicity experiments reported here. The fungus was incubated on oat-soil mixture (Padwick, 1940 *a*) for 3 weeks at 29-30° C. Weighed amounts of inoculum (5 per cent. of weight of soil taken in each pot) were added to pots and mixed with the upper 1″ layer of soil thoroughly by means of a sterilized scalpel.

4. *Micro-Nutrient Studies*

(Details of the technique used in this study and the bulk of the results accruing thereof have already been published by the author in the *Jour. Madras Univ.*, 1950. In this paper, however, some of the important results of this aspect of the problem are discussed.)

Experimental

Details of Isolates :

A number of *Fusarium* isolates were obtained from wilted plants from the field as also from wilt-sick soils and infected seeds. These strains will be referred to as follows:—

Strain I (*Fusarium udum*)—identified strain obtained from the Mycologist, Agricultural Research Institute, Coimbatore.

Strains II, III and IV (*Fusarium udum*?)—from roots of infected pigeon-pea plants (collected from Coimbatore fields).

Strain V—isolated from red gram seeds which became infected when sown in wilt-sick soil (Coimbatore).

Strain VI—was found to colonize red gram stubble buried in Coimbatore wilt-sick soil as also to infect seeds when sown in the same soil.

Strain VII—was obtained from a single attack of wilt in the laboratory garden plot (1947).

Strain VIII—from the same plot (as above) in the following year when the incidence was considerably higher.

I. *Cultural Characters of* F. udum *and Isolates*

The fungi were cultured on 2 per cent. potato-dextrose agar and steamed rice (Wollenweber, *et al.*, 1925), the former medium having been found by Padwick (1940 *b*) to be more reliable than others, especially for recording characters requiring minute microscopic examination. Examination of conidial characters were made in water mounts at a magnification of 400×. All the isolates were grown in triplicate on the above media at 30° C. Colours were recorded according to Maerz and Paul's *Dictionary of Colour* (1930) on the 15th and 20th day after inoculation. The remaining notes were made between 20 and 24 days.

Results.—It was seen as shown in Table I, that the Strains II to VIII though resembling the type strain in not producing sclerotia, yet with the exception of Strain III, varied considerably in their colour manifestation on rice. They also differed in the septation of conidia and the production of sporing structures such as pionnotes and chlamydospores. However, the conidia of the various strains did not differ much from each other when comparing breadth measurements but, as for their linear measurements the range was so wide for all the strains except Strain III that no distinct affinities or differences could be pointed out from the figures obtained.

It may be mentioned here that definite conclusions cannot be arrived at regarding the variability and even specificity of these isolates on the basis of their varying morphological and cultural characters as seen in Table I, as such characters alone are not enough to distinguish the identity of Fusaria, although they play a major part. It is, therefore, appropriate to fall in line with Wollenweber's (1938) conclusion that all cultures, highly virulent on pigeon-pea, are *F. udum*, provided "the form circle is enlarged to include those forms producing anthracene purple or similar pigments, certain forms producing no chlamydospores and forms producing no or few long septate spores".

TABLE

Cultural characters of Fusarium udum

1 Culture	2 Isolated from	3 Sclerotia	4 Septation of spores on 2% P.D.A.	5 Micro-conidia (based on 50 in μ. Length × Breadth)	6 Macroconidia—(Based on 50 in μ) (Length × Breadth)			
					1 Septate	2 Septate	3 Septate	4 Septate
Strain I.. *F. udum*	Pigeon-pea	None	1-3	6-21×1-5	16-30×3-5	28-44×3-5	28-44×3-5	..
Strain II..	Pigeon-pea (Coimbatore wilt)	None	1-3	7-17×3	24×3	28×7	34×3-7	..
Strain III..	Pigeon-pea (Coimbatore wilt)	None	1-3	7-10×1-3	14-17×1-3	21×1-3	28-34×1-3	..
Strain IV..	Pigeon-pea (Coimbatore wilt)	None	1	8-13×2-3	17×3-5	..	..	..
Strain V..	Wilt-sick Soil (Coimbatore)	None	1-4	4-17×1-5	14-17×3	24-28×3-7	31-37×5-7	41-44×5-7
Strain VI..	Wilt-sick Soil (Coimbatore)	None	1-4	6-21×3-5	17-28×3-7	28×3-7	34-37×3-5	84-44×3-5
Strain VII..	Pigeon-pea (Lab. wilt)	None	0-3	10-21×3	17-34×3-7	17-34×3-7	31-41×3-7	..
Strain VIII..	Pigeon-pea (Lab. wilt)	None	1-4	10-17×3-7	17-24×7	28-31×3-7	37-51×3-5	37-44×7
Fusarium udum Butl. (*non* Berk.)	**Cajanus* (pigeon-pea)	..	..	5-15×2-4	5-15×2-4	..	15-50×3-5	5 septate 15-50×3-5

* Data cited from Wollenweber, 1938.

II. *Virulence of* F. udum *and Isolates*

In all the trials, there were four replications for each treatment, 10 seeds being sown per pot. Only 5 per cent. inoculum by weight of soil was added to each pot as higher amounts of inoculum were found to be very destructive resulting in a high percentage of pre-emergence wilt.

I

and other Fusarium *isolates*

7 Apices of long spores	8 Colour on rice	9 Sporing structures on 2% p.d.a.	10 Chlamydospores on 2% P.D.A.
Sickle-shaped, a very few rounded	Plate 9, J_3 Plate 9, A_{12} (Yellow and orange pigment)	No characteristic sporing structures—Pale salmon pink colouration on p.d.a.	T† & I† few
Pointed	Ruby	No characteristic sporing structures (white fluffy mycelium).	None
Root-celled	Plate 9, J_3 Plate 9, A_{11} (Yellow and orange pigment)	Numerous salmon pink Pionnotes	T & I abundant
Rounded	Turtle dove (purplish)	No characteristic sporing structures (white fluffy mycelium)	T & I very few
Pointed	Turtle dove	Ivory yellow pionnotial slime	None
Rounded; 3 septate, few hooked	Plate 9, H_3; and Turtle dove (Yellow and purple)	No characteristic sporing structures; Harvard Crimson on 2% p.d.a.	T & I abundant
Pointed	Turtle dove	Creamish pionnotial masses	T & I abundant
Pointed	Turtle dove	Ivory yellow pionnotes	T and I abundant
Sickle-shaped: foot-cell present	Orange	Conidia colour up to orange; stroma colour on alkaline substratum (?) sporodochia present	T present

T† & I† = Terminal and intercallary.

The following summarises the results presented in Table II:

1. *F. udum*, Strain I, was the most virulent of all the strains, Strains II and III (isolated from wilted pigeon-pea plants collected at Coimbatore) being more pathogenic than the others.

2. Strains V and VI were responsible for the highest pre-emergence wilt figures.

TABLE II

Showing percentage pre-emergence wilt incidence and seedling wilt in infection trials with Fusarium udum *and isolates*

Fungus	Total No. of seeds	No. emerged	No. destroyed by pre-emergence wilt	No. wilted	% pre-emergence	% of seeds yielding Fusarium	% of seedling wilt
Strain I (*F. udum*)	40	23	12	16	34	67	69
Strain II ..	40	33	2	17	6	..	51
Strain III ..	40	34	1	11	3	..	32
Strain IV ..	40	32	3	2	9	..	6
Strain V ..	40	18	17	2	49	80	31
Strain VI ..	40	17	18	3	51	100	18
Strain VII ..	40	30	5	..	14	20	..
Strain VIII ..	40	31	4	2	11	..	6
Control ..	40	35	..	..	..	..	..

3. The wilted plant material and "pre-emerged" seeds in every case were surface sterilized in 1/1,000 $HgCl_2$ and plated on 2 per cent. potato dextrose agar and the *Fusarium* concerned in the incidence was obtained in all these cases.

III. *Micro-Nutrient Studies*

A. Micro-element nutrition and disease development in *Cajanus cajan.*

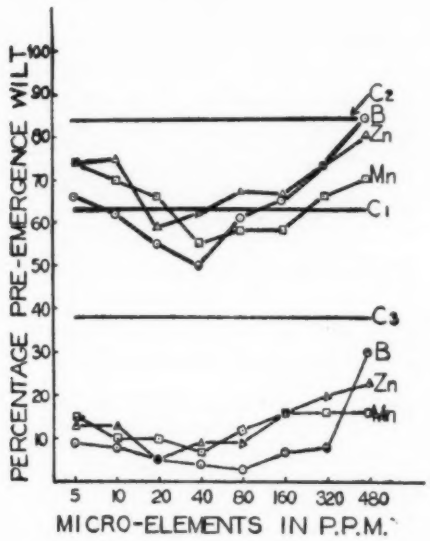
Experiment I.—The effect of boron, manganese and zinc on plant development and disease incidence in red gram was studied, and the results presented in detail earlier by Sarojini (1950). Nevertheless, some of the important results of these experiments are presented hereunder:

Results.—Text-Fig. 1 summarises the pre-emergence wilt of *Cajanus cajan* in micro-element amended wilt-sick soils.

Broadly speaking, micro-nutrient treated series on the whole yielded better results than the control, by lowering the disease index to an appreciable extent and at the same time promoting plant growth.

Of the treatments tried, manganese was most beneficial followed by zinc and boron in the decreasing order of efficiency. It is interesting to note that at the levels used, manganese and zinc did not produce any apparent toxic symptoms even with the highest dosage used in the series.

It was felt that the greater protection seen in the lower levels of the micro-elements, *viz.*, between 20 and 80 p.p.m., was due to the increased plant growth (vigour) in the presence of a more complete nutrition. To determine this, estimates of dry weight of shoots thirty days following sowing of seeds



TEXT-FIG. 1. Showing the pre-emergence wilt of *Cajanus cajan* in micro-element amended wilt-sick soils. Top bold lines represent micro-element amended November soils and bottom thin lines represent March soils along with their controls C_1, C_2, and C_3.

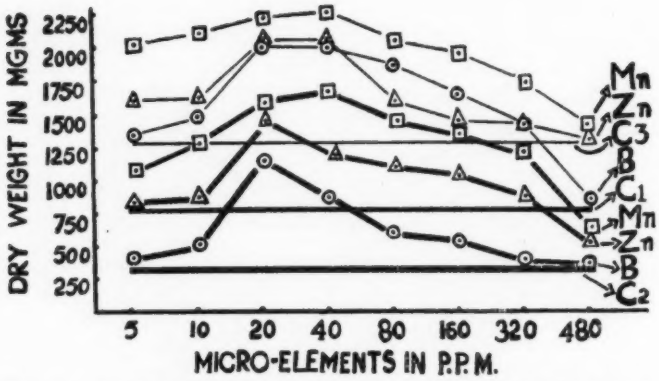
C_1 – Bare November soil.
C_2 – November soil + *F. udum* inoculum.
C_3 – Bare March soil.

were made and shoot lengths were obtained by cutting one inch below the first leaf node. The material was dried in an electric oven at 135° C. for 2 hours (Anon, 1935). The dry weight estimation of shoots belonging to the November and March trials are presented in Text-Fig. 2 (mean of 3 replicates, 5 plants for each replicate).

Results.—The treated series showed an appreciable increase in dry matter over the control, especially at the lower levels.

20 p.p.m. was optimum for boron and zinc while 40 p.p.m. level was most favourable for manganese.

A wide variation was observed between the November and March trials both in the percentage pre-emergence *wilt* and shoot weight figures, not only in the inoculated but also in the control series. The improvement seen with the March soil could be possibly due to the varying climatic and soil conditions prevalent in the two periods of soil collection, March being one of the fallow months for the crop in Coimbatore. Nevertheless, it is interesting to note



TEXT-FIG. 2. Showing the dry weight estimation of *Cajanus cajan* shoots growing in micro-element amended wilt-sick soils. Bold lines below represent micro-element amended November soils and top thin lines represent amended March soils along with their controls C_1, C_2 and C_3.

C_1 — Bare November soil.
C_2 — November soil + *F. udum* inoculum.
C_3 — Bare March soil.

that irrespective of the degree of infectivity of the soil, a distinct improvement was obtained in the micro-nutrient treated series over the control.

A further investigation on the response of the plants (growing in March soil) to combinations of the micro-nutrients at the optimum levels—20, 40 and 80 p.p.m.—revealed that "individual" micro-element nutrition was more beneficial for seed emergence and subsequent plant development in wilt-infested soil than "combined" micro-element nutrition. This may be due to the pathogen benefiting more by a supply of the nutrient solutions in combination than when added individually as, indeed, increased mat production of the wilt fungi—*F. vasinfectum*, *F. moniliforme* and *F. udum*—has been observed in pure culture by Yogeswari (1948) in the presence of boron, manganese and zinc in combination than when supplied singly.

Experiment II.—The response of the plants alone to additions of micro-nutrient solutions in combination and separately at levels of 20, 40 and 80 p.p.m. was then studied.

The heights of plants were taken after 60 days' growth in pots before they were transplanted in the field, after which observations were made to determine the effect of the initial micro-element nutrition on the normal development of the plant with special emphasis on the flowering period, germination capacity and quality of the seed produced.

TABLE III

Showing the height of red gram plants (mean of 5 replicates—3 plants for each replicate), the time taken to flower (15 plants observed for each treatment), the fresh and dry weight estimation of seeds produced (mean of 5 replicates—500 seeds in 5 lots of 100) and germination capacity of seeds (mean of 5 replicates—500 seeds in 5 lots of 100) collected from plants treated with 20, 40 and 80 p.p.m. levels of boron, manganese and zinc separately and in combination

Micro-element	Levels in p.p.m.	Height of plants	Period of flowering (Days after sowing)	Fresh weight of seeds in g.	Dry weight of seeds in g.	Percentage germination
Boron .	20	2′	143–147	7·192	6·526	80
	40	2′ 4″	146–155	7·392	6·772	76
	80	1′ 5″	149–178	7·688	6·36	65
Manganese ..	20	3′	144–145	7·722	6·923	80
	40	2′ 5″	138–144	7·7	7·054	90
	80	1′ 9″	138–144	7·964	7·109	92
Zinc ..	20	2′ 5″	148–150	7·347	6·691	90
	40	2′ 3″	146–151	7·425	6·819	92
	80	2′ 1″	141–147	7·251	6·674	92
B+Mn+Zn Combination	20	3′	130–132	7·325	6·867	80
	40	3′ 5″	120–123	7·357	6·876	88
	80	2′	135–138	7·634	7·262	86
Control ..		1′ 4″	149–155	7·275	6·564	80

The following observations can be made from Table III:

40 p.p.m. combination level seemed to be most favourable producing the tallest plants of the entire experiment. Manganese and combination treatments gave almost identical results at the 20 and 80 p.p.m. levels.

Boron was definitely harmful at the two higher levels and injury was seen in the lower leaves at those levels of the individual treatment as well as to a lesser extent in the higher levels of the combination treatment.

Flowering was earliest in the 40 p.p.m. combination treatment, the individual manganese treatment following closely. Zinc and boron treatments did not differ much from the control except that the 80 p.p.m. level of boron had a pronounced inhibitory effect on general plant development (see Sarojini, 1950, Pl. I, Fig. 2) and flowering.

The optimum figures for seed weight were obtained in the manganese treatment, the combination series yielding equally good results. Zinc was definitely better than boron which on dry weight basis gave values lower than the control at two of the three levels tried—*viz.*, at 20 and 80 p.p.m.

The germination capacity of seeds collected from micro-nutrient treated plants was, on the whole, better than the control in all the treatments except the two higher levels of boron, the individual micro-nutrient treatments yielding more favourable results than the combination series.

Experiment III.—The effect of minor elements on germination of *Cajanus* seeds was determined by soaking the seeds in various strengths of the nutrient solutions, after which counts were made of the number germinated. Controls were maintained with distilled water.

TABLE IV

Showing the percentage germination of seeds soaked for 8, 16 *and* 24 *hours in nutrient solutions of boron, zinc and manganese*

Micro-element	Levels in p.p.m.	8 hours	16 hours	24 hours
Boron ..	2	85	78	78
	5	84	81	81
	20	92	81	71
Manganese ..	2	78	78	83
	5	78	79	80
	20	78	78	81
Zinc ..	2	74	89	74
	5	85	81	78
	20	78	89	81
Control (soaked)		79	74	74
Control (unsoaked)		74		

Table IV shows a marked beneficial effect in the 16 and 24-hour soaking trials over the control. Longer periods of soaking in all levels of boron were detrimental to germination while with manganese it was the reverse. In zinc, the first two periods were more favourable than a 24-hour soaking.

B. *Micro-nutrient element and colonization and survival of the pathogen*

Experiment I.—Surface sterilized red gram stem pieces were buried in pots containing infected soil (March 1947 collection) and initial additions of the micro-elements at the three favourable levels—20, 40 and 80 p.p.m.—were made to adjust the moisture-holding capacity of the soil to 50 per cent.

Thereafter, loss in moisture was made good with necessary additions of distilled water. 25 stem pieces were buried per pot and plated out at monthly intervals. The results are presented in Table V.

TABLE V

Showing the percentage colonization of Fusarium *on red gram stubble buried in infected soil as affected by the addition of micro-nutrient solutions of boron, manganese and zinc*

Micro-element	Levels in p.p.m.	Period of incubation in months			
		1	2	3	4
Boron ..	20	20	8	8	34
	40	16	25	66	84
	80	4	20	44	76
Manganese ..	20	26	35	77	92
	40	4	20	76	88
	80	16	37	84	92
Zinc ..	20	20	34	13	..
	40	12	8	..	..
	80	12	7	..	..
Control ..		100	100	100	100

The following observations can be made from Table V:

Colonization in the control pots was 100 per cent. throughout the duration of the experiment while in the treated series, it was very low during the first few weeks and a fall in percentage colonization was noted with increase in concentration of the nutrient solution added.

Boron and manganese behaved similarly in that the colonization percentage increased gradually with increase in incubation period till at the end of four months it was almost 100 per cent. at all the levels.

Zinc, however, showed a marked inhibitory effect on colonization of the fungus which began to fall from the eighth week onwards and at the end of four months there was no colonization at the three levels.

The distinct fall in *Fusarium* colonization with addition of zinc nutrient solution compares favourably with the results obtained by Millikan (1938, 1942) in Australia where he observed a definite improvement in wheat crops attacked by several root-infecting pathogens on the addition of zinc sulphate 15-30 lb. per acre).

Experiment II.—Host stubble, infected in pure culture, was buried in garden 'compost' soil in pots and the survival of the pathogen in the presence of the micro-elements (20, 40 and 80 p.p.m. levels) was studied. Moisture holding capacity of the soil was adjusted to 50 per cent. and weekly platings (25 pieces per treatment) of the buried material were made up to two months to determine the viability of *Fusarium udum* under the above experimental conditions.

TABLE VI

Showing the percentage viability of F. udum *on red gram stubble buried in soils receiving micro-nutrient treatments*

Micro-element	Levels in p.p.m.	Period of incubation in weeks							
		1	2	3	4	5	6	7	8
Boron ..	20	96	84	72	56	32	16	12	4
	40	96	80	76	56	36	12	..	..
	80	90	80	76	62	32	8	..	..
Manganese ..	20	90	84	76	64	36	20	12	12
	40	92	84	76	64	40	24	16	4
	80	90	80	72	60	40	20	16	8
Zinc ..	20	75	57	38	12	4	..	..	..
	40	76	48	32	8	..	..	..	..
	80	75	47	30	6	..	..	..	..
Control ..		100	100	96	88	84	80	76	76

Results presented in Table VI show that with addition of micro-nutrient solutions there was considerable fall in percentage survival as compared with the control.

An appreciable difference in the behaviour of the fungus was not observed with variation in concentrations of the nutrient solutions. Zinc nutrient solutions hastened the disappearance of the fungus most rapidly, the pathogen being exterminated within four to six weeks of incubation in the soil.

DISCUSSION

The problem of pigeon-pea wilt has been left untouched almost, except for the work done at Delhi (Padwick, 1940-41). This dearth of scientific investigation is all the more striking when it is seen that in almost all the provinces where *Cajanus* is cultivated, there is a deficit in yield of more than half the amount consumed. In Delhi, it has been conclusively reported that the only practical means of combating the disease is by evolving wilt-resistant

strains, while in South India, although wilt has been found to be widely prevalent in many of the cultivated areas, yet not much has been done to estimate the extent of loss due to wilt or to bring about a control.

During the course of the present study, seven cultures of *Fusarium* distinctly varying in their morphology and virulence, were isolated from diseased *Cajanus* material and wilt-sick soils. Culturally only one of these, Strain III, resembled the type strain. Although *F. udum* was virulent by itself, yet it is possible that the high wilt incidence in cultivated areas is probably due to the association of more than one strain of *Fusarium* as this dual form of Fusarioses has already been reported in Delhi (Padwick, 1937–38).

The existence of the different isolates may be explained as due to the variability in *F. udum* as most of the strains exhibit close affinities, culturally and morphologically, with the type fungus. Indeed, variability in other species of *Fusarium* has been recorded by Haymaker (1926), Leonian (1929), Sherbakoff (1939) and Miller (1945).

Recent work on plant nutrition and disease development has shown that the susceptibility of plants, especially to the attack of certain wilt pathogens, is controlled to a great extent by their nutrition. In the present investigation it was observed that the addition of micro-nutrient elements like boron, manganese and zinc to wilt-sick soils reduced considerably the loss due to pre-emergence attack as well as wilt incidence in *Cajanus*, irrespective of the season of collection of the wilt-sick soil. Manganese yielded most beneficial results while zinc was not as satisfactory as manganese. Boron, however, was found to be definitely toxic beyond a certain concentration. Not only was disease-incidence lowered but there was also promotion of plant growth which might probably be due to the nutrient being utilized mainly by the plant resulting in better vigour and, therefore, better resistance. The addition of boron, manganese and zinc individually, or in combination, at the optimum levels, was seen to benefit the plant to the extent of early flowering and production of a better quality of seeds yielding higher germination figures. The micro-element nutrition was also effective in stimulating pigeon-pea seeds to early germination, culminating in better seedling vigour and development. The practical value of similar results when obtained in large field-scale trials is obvious as for instance this hastening in germination would probably ward off pre-emergence attack.

Manganese which yielded the best results throughout has been already recognized as a very essential element for the normal growth of plants in general (McHargue, 1922, 1926; Samuel and Piper, 1929; Marsh and Wilbur, 1945). The stimulative action of manganese on seed germination

has also been observed by several investigators (Loo and Tang, 1945 and Venkataramani, 1949).

In a study of the behaviour of the fungus in the soil with reference to its colonization and survival capacity in the presence of boron, manganese and zinc, it was seen that colonization was controlled for at least two to three months' duration by the addition of the nutrient solutions (20, 40 and 80 p.p.m. levels). Zinc was more efficient than manganese and boron in preventing *Fusarium* from colonizing on host stubble.

The results obtained with regard to survival of *F. udum* indicated that disappearance of the fungus from soils could be hastened considerably by the addition of the micro-elements, the most rapid fall in viability being obtained in the zinc treatment.

Taking into consideration the earlier findings with reference to micro-element nutrition and plant vigour, it may be stated that while manganese is essential for *Cajanus* development, the importance of zinc in bringing about greater protection from wilt may be attributed to its inhibitory influence on the saprophytic activity of the fungus in soil. A combined beneficial effect is also possible in the case of all the elements, especially with zinc and manganese. There is, further, every possibility of stimulating the micro-biological flora governing the behaviour of the fungus in the presence of modified soil conditions by amending soils with trace elements (Sulochana, 1950; Sadasivan, 1950, 51).

The results presented herein emphasize the means of bringing about a control of the disease, to some extent at least, by modifying the soil conditions with micro-element amendments and thereby limiting the saprophytic and parasitic phases of the pathogen. The implications of this approach to the solution of soil-borne fungal diseases of plants is obvious and should be thoroughly exploited to bring about a field control of the disease.

Summary

1. A number of distinctly varying *Fusarium* strains were isolated during the course of the study, from wilt-sick soils (Coimbatore) and from infested host material (wilted plants from Coimbatore and the laboratory experimental plot). Comparative cultural studies with the type culture *F. udum* and the isolates revealed that the Coimbatore strains were more closely allied to the identified strain.

2. Pathogenicity tests with *F. udum* and isolates indicated that Strain I (*F. udum*) and Strains II and III were more virulent than the other strains. Pre-emergence wilt was mainly caused by Strains V and VI.

3. A greater protection to seedling emergence in wilt-infested soils was obtained by the addition of micro-nutrient solutions of boron, manganese and zinc (especially at levels of 20, 40 and 80 p.p.m.). This beneficial effect was further seen in the increase of plant vigour, early flowering and production of better type of seeds with higher germination capacity. The pre-soaking of seeds in various concentrations of the micro-elements also induced early germination and better seedling vigour.

4. The colonization of Fusaria on host stubble was temporarily retarded by the addition of the micro-nutrient solutions, especially in the presence of zinc.

5. The survival and persistence of *F. udum* was conditioned by the presence of micro-nutrient elements in the soil, zinc being responsible for the most rapid disappearance of the fungus.

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References

Anon .. *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists.* Association of Official Agricultural Chemists, Washington, 1935.

Bose, R. D. .. "The rotation of tobacco for the prevention of wilt disease in pigeon-pea [*Cajanus cajan* (Linn.) Millsp.]," *Agric. Live Stk. India*, 1939, **6**, viii, 653–65.

Butler, E. J. .. "The wilt disease of pigeon-pea and the parasitism of *Neocosmospora vasinfecta* Smith," *Mem. Dept. Agric. India*, Bot. Ser., 1910, **2**, 1–62.

——— .. "The Wilt Diseases of Cotton and Sesamum in India," *Agric. Journ. India*, 1926, **21**, 273.

Garrett, S. D. .. "Soil conditions and root-infecting fungi," *Biol. Rev.*, 1938, **13**, 159–85.

——— .. *Root Disease Fungi*, Chronica Botanica Co., Waltham, Mass., U.S.A., 1944.

Haymaker, H. H. .. "Pathogenicity of two strains of tomato wilt fungus, *Fusarium lycopersici* Sacc.," *J. agric. Res.*, 1928, **36**, 675–93.

Keen, B. A., and Raczkowski, H. "The relation between the clay content and certain physical properties of a soil," *J. agric. Sci.*, 1921, **11**, 441–50.

King, C. J., Hope, C., and Eaton, E. D. "Some microbiological activities affected in manurial control of cotton root rot," *J. agric. Res.*, 1934, **49**, 1093–1107.

Leonian, L. H. .. "Studies on the variability and dissociations in the Genus *Fusarium*," *Phytopath.*, 1929, **19**, 753–868.

Loo, T. L., and Tang, Y. M. "Growth stimulation by manganese sulphate, indole-3-acetic acid and colchicine in the seed germination and early growth of several cultivated plants," *Amer. J. Bot.*, 1945, **32**, 106–14.

Maerz, A., and Paul, M. R. *A Dictionary of Colour*. McGraw-Hill Book Company, New York, I Edn., 1930.

Marsh, A. W., and Wilbur, L. P. "Responses of plants to additions of manganese to some Oregon soils," *Amer. Soc. Agron.*, 1945, **37**, 1–8.

McHargue, J. S. .. "The role of manganese in plants," *J. Amer. Chem. Soc.*, 1922, **44**, 1592–8.

———— .. "Manganese and plant growth," *J. Indust. Engng. Chem.*, 1926, **18**, 172.

McRae, W., and Shaw, F. J. F. .. "Influence of manures on the wilt disease of *Cajanus indicus*, and the isolation of types resistant to the disease," *Sci. Monogr. Counc. agric. Res. India*, 1933, **7**.

Millard, W. A., and Taylor, C. B. .. "Antagonism of micro-organisms as the controlling factor in the inhibition of Scab by green manuring," *Ann. appl. Biol.*, 1927, **14**, 202–15.

Miller, J. J. .. "Studies on the *Fusarium* of muskmelon wilt : 1. Pathogenic and cultural studies with particular reference to the cause and nature of variation in the causal organism," *Canad. J. Res.*, Sect. C, 1945, **23**, 16–43.

Millikan, C. R. .. "A preliminary note on the relation of zinc to disease in cereals," *J. Dept. Agric. Vict.*, 1938, **36**, 409–16.

———— .. "Studies on soil conditions in relation to root-rot of cereals," *Proc. Roy. Soc. Vict., N. S.* 1942, **54**, 145–95.

Mitchell, R. B., Hooton, D. R., and Clark, F. E. "Soil bacteriological studies on the control of *Phymatotrichum* root-rot of cotton," *J. agric. Res.*, 1941, **63**, 535–47.

Mitra, M. .. "Wilt disease of *Crotalaria juncea* L. (Sann Hemp.), *Ind. J. agric. Sci.*, 1934, **4**, 701–14.

Mundkur, B. B. .. "The influence of temperature and maturity on the incidence of Sann Hemp and pigeon-pea wilt at Pusa," *Ibid.*, 1935, **5**, 609–18.

Padwick, G. W. .. "Report of the Imperial Mycologist, Reports of the Indian Agricultual Research Institute, New Delhi", 1937–38, pp. 108–15; 1939–40, pp. 94–101.

———— .. "The genus *Fusarium*, III. A critical study of the fungus causing wilt of gram (*Cicer arietinum* L.) and of the related species in the sub-section Orthocera with special relation to variability of key characteristics," *Ibid.*, 1940 *a*, **10**, 241–84.

———— .. "The genus *Fusarium*, V. *F. udum* Butler, *F. vasinfectum* Atk. and *F. lateritium* Nees var. *uncinatum* Wr." *Ibid.*, 1940 *b*, **10**, 863–73.

———— .. "Report of the Imperial Mycologist, Reports of the Indian Agricultural Research Institute, New Delhi," 1940–41. pp. 52–56.

Plymen, F. J. .. *Reports on the Working of the Department of Agriculture of the Central Provinces for the years ending the 31st March* 1932 *and the 31st March* 1933, pp. 40.

Pryor, D. E. .. "The effect of some mineral nutrients on the development of club root of crucifers," *J. agric. Res.*, 1940, **61**, 149–60.

Rawlins, T. E. .. *Phytopathological and Botanical Research Methods*," John Wiley, London, 1933.

Reinking, O. A. .. "Parasitic and other Fusaria counted in Costa Rica and Panama soils," *Zbl. Bakt. Abt.*, 1934, 2, **90**, 4-17. (Cited from Garrett, 1944.)

———— .. "Soil and *Fusarium* diseases," *ibid.*, 1935, 2. **91**, 243–55. (Cited from Garrett, 1944.)

—— and Manns, M. M. .. "Parasitic and other *Fusaria* counted in tropical soils," *Z. Parasitenk.*, 1933, **6**, 23–75. (Cited from Garrett, 1944.)

———— .. "Parasitic and other *Fusaria* counted in Colombia and other soils," *Zbl. Bakt. Abt.*, 1934, 2, **89**, 502–9. (Cited from Garrett, 1944.)

Sadasivan, T. S. .. "Succession of fungi decomposing wheat straw in different soils with special reference to *Fusarium culmorum*," *Ann. appl. Biol.*, 1939, **26**, 497–508.

———— .. "Some aspects of soil-borne fungal diseases of plants" "The Palæobotanist," Birbal Sahni Memorial Volume (In Press), 1950.

———— .. "Role of trace elements in control of root-infecting fungi," *Proc. Indian Acad. Sci.*, 1951 (In Press).

Samuel, G., and Piper, G. S. "Manganese as an essential element for plant growth," *Ann. appl. Biol.*, 1929, **16**, 494–524.

Sanford, G. B. .. "Some factors affecting the pathogenicity of *Actinomyces scabies*," *Phytopath.*, 1926, **16**, 525–47.

Sarojini, T. S. .. "Soil conditions and root diseases. II. Micro-nutrient element and disease development by *Fusarium udum* on red gram," *Jour. Madras Univ.* Sect. B, 1950, **19**, 1–32.

Shear, G. M., and Wingard, S. A. "Some ways by which nutrition may affect severity of disease in plants," *Phytopath.*, 1944, **34**, 603–05.

Sherbakoff, C. D. .. "Variability in *Fusarium vasinfectum*—Abst." *Phytopath.*, 1939, **29**, 1-26.

Smith, P. G., and Walker, J. C. .. "Certain environal and nutritional factors affecting *Aphanomyces* root-rot of garden pea," *J. agric. Res.*, 1941, **63**, 1–20.

Subramanian, C. V. .. "Some factors affecting the growth and survival of *Fusarium vasinfectum* Atk. the cotton wilt pathogen in the soil, with special reference to microbiological antagonism," *Jour. Ind. Bot. Soc.*, 1946, **25**, 89–101.

Sulochana, C. B. .. "Soil conditions and wilt diseases in plants, with special reference to colonisation and survival of *Fusaria* in soils treated with micro-elements." (Thesis approved for the Degree of Master of Science, University of Madras, 1950).

Uppal, B. N., and Kulkarni, N. T. — "Studies in the *Fusarium* wilt of Sann Hemp. I. The physiology and biology of *Fusarium vasinfectum*," *Ind. J. agric. Sci.*, 1937, **7**, 413–42.

Venkataramani, K. S. .. — "Studies on Indian Vegetables." (Thesis approved for the Degree of Doctor of Philosophy, University of Madras, 1949).

Waksman, S. A. .. — "Is there any fungus flora of the soil?" *Soil Sci.*, 1917, **3**, 565–89.

Walker, A. G. .. — "The colonisation of buried wheat straw by soil fungi with special reference to *Fusarium culmorum*," *Ann. appl. Biol.*, 1941, **28**, 333–50.

Wollenweber, H. W. .. — "Fusariosen des katjangs, *Cajanus indicus* (Fusarioses of pigeon-pea, *Cajanus indicus*)," *Arb. Biol. Anst.* (*Reichsanst Berl*, 1938, **22**, 339–47.

———, Sherbakoff, C. D., Reinking, O. A., Johann, H. and Bailey, A. S. — "Fundamentals for taxonomic studies of *Fusarium*," *J. agric. Res.*, 1925, **30**, 833–43.

Yogeswari, L. .. — "Trace element nutrition of fungi. I. The effect of boron, zinc and manganese on *Fusarium* species," *Proc. Indian Acad. Sci.*, 1948, **28**, B, 177–201.

Young, V. H. .. — "Control of cotton wilt and *rust* or potash hunger, by the use of potash-containing fertilizers," *Bull. Ark. agric. Expt. Stat.*, 1938, **358**. (Cited from Garrett, 1944.).

STUDIES IN CROP PHYSIOLOGY

Effect of Farmyard Manure and Castor Cake upon Development, Yield and Seed Quality of Wheat

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Introduction

Studies on the importance of organic manures to soil and plants have indicated the useful effect of such manures on chemical and biological changes in soil,[10,11,14,27] in stabilising soil colloidal properties,[2] in bringing about changes in soil microflora,[3,32] in increasing carbon dioxide content,[31] in altering solubility of some inorganic soil constituents[4,26,34] and in inducing buffer action in soils.[26] Their application under field conditions is also known to induce changes in the lignin humus complex[13] but no relation with soil acidity is recorded. Maintenance of proper physical condition and of nitrogen and organic matter is claimed to be brought about in three ways, *viz.*, application of plant residues, growing of legumes and green manure crops and application of organic manures.[28,29] Attempts to elucidate the changes that these organic manures undergo in soil have been made by a number of workers[1,3,5,6,7,8,9,12,15–19,21,22,25,26,28,29,33] and valuable evidences put forward to show how nitrogen and organic matter conservation are of vital importance to the maintenance of optimum soil conditions for plant growth.

On the practical side of crop production, high yield and better quality of crop have been very often obtained in response to organic manures, though the effects of organic matter as such upon yield of grain, maturity and disease resistance are considered to be of doubtful significance.[20,24] Richardson and Gurney[23] as a result of extensive investigations for over five years have shown that certain forms of nitrogen are better than others but as a general rule, the larger the quantity of nitrogen the greater were the yields of the crop. Experiments conducted under pot cultures by Singh, Lal and others[30] again showed that the relative response to different forms of organic manures and fertilisers varied with the dose of the fertilisers. Marked changes in yield and protein content of grain were also recorded under these conditions.

The present investigation is, therefore, in continuation of the enquiries under pot cultures; it is proposed to present herein the results obtained on

the relative effects of two forms of commonly used organic manures—farmyard manure and castor cake—when applied at three distinct nitrogen levels of 0, 30 and 60 lb. N per acre. Growth behaviour of two varieties of wheat, Pusa 4 and Pusa 52, was studied under these conditions of nutrition in field. Response to such manurial applications has been measured in terms of external changes in growth, yield of economic produce, and nitrogen and ash content of grain.

Plant of Experiment

The investigation was conducted on the Experimental Farm of the College. A plot of land of known previous history was selected. Sugarcane and wheat were the two principal crops grown earlier on this field for several years in succession. Green manuring with sannhemp in between the two successive crops was done prior to 1938. No manures of any form were added between 1939–43. In the cropping season of 1943–44, the field was well prepared and sown with two varieties of wheat—Pusa 4 and Pusa 52—as per layout plan of experiment (Fig. 1), the details of which are given below:—

P4 C.C. 0 lbs.	P4 F.Y.M. 30 lbs.	P4 C.C. 60 lbs.
P4 F.Y.M 30 lbs.	P52 F.Y.M. 0 lbs.	P4 F.Y.M. 0 lbs.
P52 F.Y.M. 0 lbs	P4 C.C. 0 lbs.	P52 F.Y.M. 30 lbs.
P 52 C.C. 0 lbs.	P52 C.C. 60 lbs.	P52 C.C. 30 lbs.
P 52 C.C. 60 lbs.	P4 C.C. 30 lbs.	P4 C.C. 30 lbs.
P4 C.C. 30 lbs.	P4 F.Y.M 0 lbs.	P4 C.C. 0 lbs.
P4 F.Y.M. 60 lbs.	P 52 F.Y.M. 60 lbs.	P52 F.Y.M. 0 lbs.
P4 C.C. 60 lbs.	P52 F.Y.M. 30 lbs.	P52 F.Y.M. 60 lbs.
P52 C.C 30 lbs	P4 F.Y.M. 60 lbs.	P4 F.Y.M. 60 lbs.
P52 F.Y.M. 60 lbs.	P52 C.C. 0 lbs.	P52 C.C. 60 lbs.
P4 F.Y.M. 0 lbs.	P52 C.C. 30 lbs.	P52 C.C. 0 lbs.
P52 F.Y.M. 30 lbs.	P4 C.C. 60 lbs.	P4 F.Y.M. 30 lbs.

Fig. 1. Layout plan of experiment.

Varieties.—Pusa 4 and Pusa 52.

Manures.—(i) Farmyard manure and (ii) Castor cake.

Levels of manuring.—(i) 0 lb. N; (ii) 30 lbs. N; and (iii) 60 lbs. N per acre.

Total no. of treatments.—2×2×3 or 12 only.

System of replication.—Three randomised blocks of 12 treatments each.

Plot size.—Gross plot size with borders 1/39 acre.
Net plot size .. .. 1/53 acre.

Manuring.—In full dose before sowing.

Sowing.—3rd November 1943.

Harvesting.—14th April 1944.

Two irrigations were given to the experimental plots in November and December, 1943.

Growth characters such as height, tillering, green leaf number and ear characteristics were recorded on six plants selected at random from each plot; the ear and leaf characters were noted on the main shoot. Observations at successive time intervals were conducted throughout the life-cycle of the two varieties. Finally, yield of grain and straw per plot and absolute weight of 200 grains were also found out. Only the summary tables of statistically analysed data are presented in the text. At harvest, requisite quantity of grain was taken from a composite mass collected from all replications treatment-wise, and crushed in a laboratory mill. Flour obtained was used for the estimation of moisture content, ash percentage and total nitrogen. Moisture content was determined by drying a known sample in an oven at 105° C. Ash on incineration at red heat was weighed and expressed as percentage of fresh weight of the flour. Total nitrogen in flour was estimated by Kjeldahl's method modified by Gunning to include nitrate nitrogen (Cp. "Official and Tentative Methods of Chemical Ananlysis," A.O.A.C., Washington, 1935).

Experimental Findings

A. Height of shoot as affected by forms and levels in wheat

Statistical analysis of the data recorded under different treatments showed the effect of varieties to be predominantly significant at all periods of the life-cycle (Table I). Barring the earliest stage of observation (37 days), manures and doses too, indicated significant responses at successive stages. First and higher order interactions, on the other hand, exhibited significant effects at only certain specified periods. Thus, interaction between manures and variety, showed significant responses at six stages out of a total of eight; these were at 37, 51, 67, 111, 126, and 157 days in the life-cycle. Variety × doses interaction, on the contrary, exhibited significant effects only towards

TABLE I

(*i*) *Height of main shoot of wheat plant (cm.)*

Analysis of variance

Due to	D.F.	Mean sum of squares at various stages							
		37	51	66	81	97	111	126	157
Blocks	2	0·72*	1·03	22·51	24·31	80·4	83·0*	32·8	24·6
Varieties	1	10·62*	152·83*	1977·44*	4037·12*	342·2*	1·9*	129·2*	455·7
Manures	1	0·09	11·34*	288·47*	1668·82*	550·4*	6598·5*	802·2*	634·6*
Doses	2	0·08	23·96*	385·10*	869·97*	675·0*	534·3*	749·4*	501·6*
Man. × Var.	1	0·89*	4·17*	27·13*	15·37	7·3	69·0*	125·9*	112·3*
Var. × Doses	2	0·04	7·44	8·93	3·06	71·2*	85·2*	92·5*	68·4*
Man. × Doses	2	0·21	1·12	38·78*	116·18*	183·0*	147·7*	152·1*	216·0*
Man. Var. Doses	2	0·12	19·02*	0·40	26·36	47·9*	199·4*	279·7*	31·5
Error	22	0·12	1·59	11·81	24·10	18·0	23·1	21·9	17·8
Total	35	..	..	..	..	..	..	..	..
S.E./Observation		±0·35	±1·26	±3·44	±2·21	±4·24	±4·80	±4·9	±4·2

* Significant effects.

(*ii*) *Man. × Var. and Man. × Doses Interaction*

Age	Manures	Varieties		Doses			Mean	Standard difference 5%
		Pusa 4	Pusa 52	0 lb.	30 lb.	60 lb.		
37	F. Y. Man.	4·60	3·76	4·18	4·17	4·19	4·18	Var. or Man. ±0·242
	Castor C.	5·07	3·74	4·32	4·38	4·52	4·40	
	Mean	4·83	3·75	4·25	4·27	4·36	..	Doses ±0·295
52	F. Y. Man.	13·03	9·60	10·25	10·84	12·85	11·32	Var. or Man. ±0·871
	Castor C.	14·80	10·36	10·82	12·63	13·51	12·32	
	Mean	13·94	9·98	10·53	11·74	13·18	..	Doses ±1·068
67	F. Y. Man.	38·16	25·80	27·80	29·30	37·77	31·62	Var. or Man. ±2·376
	Castor C.	44·94	28·80	29·55	38·24	42·20	36·66	
	Mean	41·55	26·94	28·67	32·77	39·97	..	Doses ±2·912
82	F. Y. Man.	71·95	51·97	56·89	60·01	68·98	61·95	Var. or Man. ±3·388
	Castor C.	81·74	59·38	58·33	73·11	80·25	70·56	
	Mean	76·84	55·67	57·61	66·56	74·61	..	Doses ±4·150
97	F. Y. Man.	99·75	94·88	93·41	98·02	100·50	97·32	Var. or Man. ±2·933
	Castor C.	108· 8	101·40	92·30	108·90	114·16	105·22	
	Mean	104· 2	98·14	92·86	103·46	107·33	..	Doses ±3·592
111	F. Y. Man.	100· 0	98·20	96·00	99·12	102·36	99·16	Var. or Man. ±3·322
	Castor C.	109· 4	105·05	94·94	110·81	117·36	107·70	
	Mean	104· 7	102·70	95·47	104·47	109·86	..	Doses ±4·007
126	F. Y. Man.	100·54	98·50	97·05	99·41	103·11	99·52	Var. or Man. ±3·442
	Castor C.	111·65	106·20	94·61	111·29	120·80	108·90	
	Mean	107·09	102·35	95·83	105·33	111·45	..	Doses ±4·21
156	F. Y. Man.	101·08	99·00	97·16	100·61	102·40	100·65	Var. or Man. ±2·92
	Castor C.	110·65	107·05	97·66	110·60	117·46	108·57	
	Mean	105·86	103·02	97·63	105·60	109·93	..	Doses ±3·575

the later stages of 97, 111, 126 and 157 days. Interaction due to manures × doses was again significant at stages on or beyond 66 days in the life-cycle. Higher order interaction between manures, doses, and varieties, however, showed significant effects at 51, 97, 111 and 126 days in the growth cycle of this crop.

Pusa 4 at majority of stages showed significantly superior height than Pusa 52. Similarly castor cake proved uniformly better than farmyard manure in improving the stand of the crop. Increasing additions of these manures had a marked augmentative effect on this character inasmuch as each successive addition upto 60 lb. N level resulted in significant increase in height. This was true for all stages of growth after 52 days. In general, augmentative effects of such manurial applications were felt only after the lapse of a certain period during which the organic nitrogen was slowly rendered available for the growth of the crop.

B. Tillering in response to forms, levels and varieties

Analysis of data showed that varieties had less significant effect on tillering. Only at 52 and 82 days in the life-cycle, were the main effects due to varieties significant. Manures, on the contrary, proved significant at 37, 67, 97 and 111 days. Doses were, however, found to significantly influence tillering at 37, and 111 days only. First order interaction between manures × varieties proved to be more effective on tillering than others. Tillering was significantly affected in this case at all stages beyond 52 days in the life-cycle (Table II). Variety × dose interaction was effective only at one stage (97 days); interaction between manure and dose was on the contrary significant at 37, 97 and 111 days. In contrast to these, the higher order interactions between the three factors showed significant responses at the earliest stage of observation, *viz.*, 37 days only. Difference between varieties, or between doses of fertilisers were, barring few exceptions, largely insignificant. Castor cake again proved to be more effective than farmyard manure, differences between the two being statistically significant at four out of six stages in the life-cycle.

C. Effect of forms, levels and varieties on green leaf number

Main effects due to variety was significant at 52, 82 and 97 days in the life-cycle (Table III). Manures too showed marked effects at 67 and 97 days. Doses, on the contrary, showed significant responses at intermediate periods of 52 and 67 days. All first and higher order interactions, on the contrary, did not show any significant effect at all. On number of green leaves on main shoot none of the factors—varieties, doses or forms—showed any consistent

effect. In majority of cases, the differences were not significant at all. Wherever significant effects were noted, the specific responses of either the varieties

TABLE II

(i) Total number of tillers per plant

Analysis of variance

Due to	D.F.	Mean sum of squares at various stages (days)					
		37	52	67	82	97	111
Blocks	2	1·10*	2·36*	2·93*	4·21*	4·24*	6·31*
Varieties	1	0·002	2·60*	0·73	2·93*	0·31	0·30
Manures	1	0·11*	0·57	1·27*	2·45	1·48*	2·87*
Doses	2	0·17*	0·10	0·38	0·39	0·31	1·61*
Man. × Var.	1	0·03	2·628*	2·43*	3·41*	6·19*	3·42*
Var. × Doses	2	0·06	0·23	0·02	0·97	0·86*	0·002
Man. × Doses	2	0·16*	0·05	0·04	0·39	0·94*	1·44*
Man. × Var. × Doses	2	0·16*	0·02	0·37	0·45	0·69	0·53
Error	22	0·03	0·54	0·61	1·45	0·41	0·38
Total	35	..	..	..	..	..	..
S.E./Observation		±0·17	±0·73	±0·78	±1·20	±0·64	±0·61

* Significant effects.

(ii) Man. × Var. and Man. × Doses Interaction

Age	Manures	Varieties		Doses			Mean	Standard difference 5%
		Pusa 4	Pusa 52	0 lb.	30 lb.	60 lb.		
37	F. Y. Man.	3·32	3·36	3·35	3·50	3·23	3·36	Var. or Man. ±0·122
	Castor C.	3·52	3·44	3·265	3·54	3·63	3·48	
	Mean	3·421	3·401	3·307	3·52	3·42	..	Doses ±0·150
52	F. Y. Man.	6·41	7·50	7·01	6·93	6·90	6·94	Var. or Man. ±0·051
	Castor C.	7·22	7·14	7·10	7·13	7·21	7·14	
	Mean	6·815	7·32	7·05	7·03	7·05	..	Doses ±0·062
67	F. Y. Man.	6·67	7·49	7·16	6·98	7·10	7·08	Var. or Man. ±0·541
	Castor C.	7·53	7·14	7·00	7·38	7·63	7·34	
	Mean	7·07	7·31	7·08	7·18	7·36	..	Doses ±0·664
82	F. Y. Man.	6·22	6·84	6·58	6·50	6·51	6·53	Var. or Man. ±0·883
	Castor C.	7·54	6·56	6·71	6·95	7·50	7·05	
	Mean	6·88	6·70	6·65	6·73	6·00	..	Doses ±1·020
97	F. Y. Man.	4·55	5·42	5·23	4·76	5·02	5·00	Var. or Man. ±0·444
	Castor C.	5·81	4·93	5·15	5·13	5·83	5·37	
	Mean	5·18	5·17	5·19	4·95	5·42	..	Doses ±0·543
111	F. Y. Man.	4·44	4·89	5·01	4·18	4·80	4·66	Var. or Man. ±0·0415
	Castor C.	5·62	4·83	4·78	5·03	5·86	5·22	
	Mean	5·03	4·86	4·89	4·60	5·33	..	Doses ±0·052

TABLE III

(i) Total number of green leaves on the main shoot

Analysis of variance

Due to	D.F.	Mean sum of squares at different stages (days)				
		37	52	67	82	97
Blocks	2	0·088	0·038	0·254	0·178	0·358*
Varieties	1	0·054	0·141*	0·095	0·174*	0·647*
Manures	1	0·027	0·048	0·236*	0·295	0·530*
Doses	2	0·032	0·114*	0·312*	0·108	0·334
Man. × Var.	1	0·044	0·010	0·500	1·751*	0·031
Var. × Dose	2	0·007	0·035	0·003	2·508*	0·032
Man. × Dose	2	0·005	0·016	0·099	0·040	0·011
Var. × Man. × Dose	2	0·017	0·036	0·030	0·082	0·009
Error	22	1·128	0·041	0·061	0·818	0·036
Total	35	..	..	..	..	..
S.E./observation		±0·350	±0·203	±0·247	±0·904	±0·189

* Significant effects.

(ii) Man. × Var. and Man. × Doses Interaction

Age	Manures	Varieties		Doses			Mean	Standard difference 5%
		Pusa 4	Pusa 52	0 lb.	30 lb.	60 lb.		
37	F. Y. Man.	2·21	2·16	2·13	2·18	2·25	2·188	Var. or Man. ±0·156
	Castor C.	2·21	2·14	2·21	2·11	2·20	2·177	
	Mean	2·21	2·15	2·17	2·14	2·22	..	Doses ±0·19
52	F. Y. Man.	2·96	2·87	2·96	2·80	3·00	2·921	Var. or Man. ±0·009
	Castor C.	2·99	2·83	2·91	2·83	3·01	2·965	
	Mean	2·98	2·85	2·94	2·81	3·07	..	Doses ±0·014
67	F. Y. Man.	4·42	4·43	4·25	4·36	4·65	4·544	Var. or Man. ±0·170
	Castor C.	4·66	4·57	4·53	4·66	4·66	4·621	
	Mean	4·54	4·50	4·39	4·51	4·65	..	Doses ±0·209
82	F. Y. Man.	3·66	4·16	3·91	3·95	3·99	3·912	Var. or Man. ±0·624
	Castor C.	3·71	4·42	4·03	4·15	4·01	4·054	
	Mean	3·69	4·29	3·97	4·05	3·95	..	Doses ±0·766
97	F. Y. Man.	2·70	3·09	2·916	2·98	2·80	2·899	Var. or Man. ±0·041
	Castor C.	2·688	3·05	2·65	2·85	3·06	2·850	
	Mean	2·69	3·05	2·78	2·91	2·93	..	Doses ±0·048

or the doses were inconsistent. This factor for plant development, therefore, appears to be least affected by any of the factors under consideration.

D. Effect of forms, levels and varieties upon ear and grain characters

On the size of the ear, main effects due to varieties and higher order interactions between variety, manures and doses were only found to be significant. Pusa 4 appeared to be significantly superior to Pusa 52. Differences due to doses or forms of manure were not significant at all. Spikelets in the ear were, on the other hand, significantly affected under all the main effects and interactions (Table IV). Pusa 52 was better in this regard than Pusa 4. Doses and manures failed to show any marked effect.

Total number of grains in the ear responded significantly to varieties and doses. All interactions except that due to manures and doses were significant. Pusa 52 again was superior to Pusa 4. First increase of 30 lbs. had significant effect on this character. Manures were without any effect. Weight of grains per ear did not vary significantly at all in response to any of the three factors; differences between varieties, doses or forms of manure failed to reach the level of significance. So far as the absolute weight is concerned, main effects of varieties were only found to be significant. Seeds of Pusa 4 were better in this regard than those of Pusa 52. Effect of first 30 lbs. application was also significantly useful in increasing the absolute weight of seed. Forms of manure were not at all effective in this regard (Table IV).

E. Effect of forms, levels and varieties upon yield

While the main effect of variety was not significant either on grain or straw, it was so in case of total weight of grain and straw both. Manures and doses were uniformly significant. On grain yield, all first and higher order interaction proved to be significant. On straw, manure × variety, and manures × doses interactions only were significant (Table V). Total produce was also significantly affected in response to variety × doses, and manures × doses interactions. Higher order interactions were without any effect.

Differences between the two varieties and between the forms of manure were highly significant. Pusa 52 and castor cake proved to be definitely better. Successive additions of manure also increased yield significantly. There was, however, no difference between the two varieties in so far as yield of straw was concerned. Castor cake again proved better than farmyard manure. The effects under 30 lb. dose were highly significant; higher doses were not effective in improving straw yield. Total produce, on the other hand, was again better under castor cake though varieties did not exhibit significant variations (Table V). Application at 30 lbs. level only proved to be of use in improving yield of total produce.

TABLE IV

(i) Ear and grain characteristics of wheat under different treatments

Analysis of variance

Due to	D.F.	Ear size	Spikelets in ear	No. of grains in ear	Wt. of grains in ear	Abs. wt.
		Mean sum of squares				
Blocks ..	2	0·49	46·32*	94·09*	0·620*	1·84*
Varieties ..	1	8·60*	13·40*	197·30*	0·019	6·25*
Manures ..	1	0·33	7·36*	9·15	0·001	0·50
Doses ..	2	0·80	3·58*	21·87*	0·067	0·01
Man. × Var. ..	1	0·26	6·48*	27·07*	0·013	0·27
Var. × Doses ..	2	0·22	2·61*	29·56*	0·035	0·42
Man. × Doses	2	1·03	3·30*	1·69	0·015	0·01
Var. × Man. × Doses ..	2	2·94*	6·37*	155·38*	0·656*	0·15
Error ..	22	0·73	1·05	5·33	0·102	0·26
Total ..	35	..	..	..	..	..
S.E./observation ..	..	±0·85	±1·024	±2·30	±0·31	±0·515

* Significant effects.

(ii) Man. × Var. and Man. × Doses Interaction

Manures	Varieties		Doses			Mean	Standard difference at 5%
	Pusa 4	Pusa 52	0 lb.	30 lb.	60 lb.		
	Length of the main shoot ear in cm.						
F. Y. Man. ..	8·71	8·67	8·29	7·80	8·47	8·190	Var. or Man.
Castor C. ..	8·85	7·78	7·80	8·65	8·31	8·256	±0·591
Mean ..	8·78	7·72	8·04	8·22	8·39	..	Doses ±0·724
	Number of spikelets in the ear of main shoot						
F. Y. Man. ..	19·57	21·69	20·33	20·66	20·95	20·65	Var. or Man.
Castor C. ..	21·31	21·25	22·47	20·75	20·63	21·285	±0·700
Mean ..	20·44	21·47	21·40	20·70	20·79	..	Doses ±0·867
	Number of grains in the ear of main shoot						
F. Y. Man. ..	38·33	44·65	40·43	42·25	42·00	41·56	Var. or Man.
Castor C. ..	41·05	43·96	40·55	43·56	43·41	42·51	±1·560
Mean ..	30·69	44·31	40·49	42·90	42·70	..	Doses ±1·953
	Total wt. of grains in the ear						
F. Y. Man. ..	1·40	1·50	1·40	1·45	1·50	1·45	Var. or Man.
Castor C. ..	1·43	1·46	1·34	1·50	1·50	1·44	±0·22
Mean ..	1·41	1·48	1·37	1·47	1·50	..	Doses ±0·270
	Absolute wt. of 200 grains						
F. Y. Man. ..	7·97	7·13	7·33	7·78	7·54	7·551	Var. or Man.
Castor C. ..	7·600	6·97	6·945	7·53	7·39	7·288	±0·355
Mean ..	7·788	7·053	7·13	7·65	7·46	..	Doses ±0·435

TABLE V

(i) Yield of grain and straw at harvest per plot in seers

Analysis of variance

Due to	D.F.	Wt. of grain per plot	Wt. of straw per plot	Total wt. straw and grain per plot
		Mean sum of squares		
Blocks ..	2	20·40*	107·00*	210·13*
Varieties ..	1	3·00*	11·11	124·49*
Manures ..	1	11·00*	265·58*	485·26*
Doses ..	2	20·00*	234·26*	177·57*
Man. × Var. ..	1	34·00*	44·36*	45·92
Var. × Doses ..	2	20·50*	31·62	307·70*
Man. × Doses ..	2	20·50*	34·17*	101·73*
Var. × Man. × Doses ..	2	6·50*	11·42	2·56
Error ..	22	2·09	16·50	39·90
Total ..	35	..	..	..
S.E./plot ..	..	±1·445	±4·006	±6·300

* Significant effects.

(ii) Man. × Var. and Man. × Doses Interaction

Manures	Varieties		Doses			Mean	Standard difference 5%
	Pusa 4	Pusa 52	0 lb.	30 lb.	60 lb.		
			Wt. of grain per plot				
F. Y. Man. ..	6·66	9·333	7·00	7·50	9·50	8·00	Var. or Man.
Castor C. ..	9·33	10·33	7·00	10·50	12·00	9·83	±0·997
Mean ..	8·00	9·83	7·00	9·00	10·75	..	Doses ±1·215
			Wt. of straw per plot				
F. Y. Man. ..	20·11	23·56	19·05	22·06	24·41	21·84	Vnr. or Man.
Castor C. ..	27·233	27·03	20·10	29·71	31·05	26·95	±2·808
Mean ..	23·67	25·29	19·57	25·89	27·73	..	Doses ±3·439
			Total wt. of grain and straw				
F. Y. Man. ..	26·48	32·93	26·06	29·90	33·66	29·87	Var. or Man.
Castor C. ..	36·70	37·44	27·50	40·50	43·225	37·37	±4·366
Mean ..	31·59	35·19	26·78	35·20	38·44	..	Doses ±5·346

F. Effect on grain composition

Recorded observations show the general tendency of high moisture content in response to the application of manures, the first dose of 30 lb. bringing about greater changes than the next higher dose (Table VI). Differences due to either varieties or forms were less characteristic. So far

TABLE VI

Moisture, ash and nitrogen percentage of grain

Doses	Farmyard Manure		Castor Cake	
	Pusa 52	Pusa 4	Pusa 52	Pusa 4
	Moisture percentage			
0 lb. ..	3·53	4·33	3·47	6·07
30 lb. ..	6·33	5·30	5·87	7·60
60 lb. ..	9·20	7·33	6·93	4·73
	Ash percentage			
0 lb. ..	3·5	5·0	2·80	2·90
30 lb. ..	2·80	5·10	2·50	2·20
60 lb. ..	3·80	4·80	2·70	2·50
	Total nitrogen × 6·25			
0 lb. ..	6·875	6·213	5·731	6·180
30 lb. ..	10·400	9·790	10·975	10·185
60 lb. ..	13·300	12·013	13·650	11·500

as ash percentage was concerned, for each level, farm-yard manure appeared to be better than castor cake. Varietal differences under castor cake were less evident than under farm-yard manure. The higher the dose, the greater was the nitrogen content or crude protein content. Under higher doses, Pusa 52 was better than Pusa 4. Differences between manures were less characteristic.

DISCUSSION

A. Effect of form of manure

From the perusal of the observations recorded in the previous pages, it would be evident that castor cake under the conditions of the experiment was undoubtedly better than farm-yard manure. This was specially noticeable in case of height of main shoot which was significantly improved over farm-yard manure at all stages of the life-cycle. Significant increases in tillering were recorded at 37, 72, 97 and 112 days, and in green leaf number at 52 days only. The general vegetative vigour of wheat varieties thus, was better under castor cake than under farm-yard manure. Judged by this and the increased yield of straw and grain under this treatment, castor cake appears to be more desirable than farm-yard manure in the production of wheat (Fig. 2).

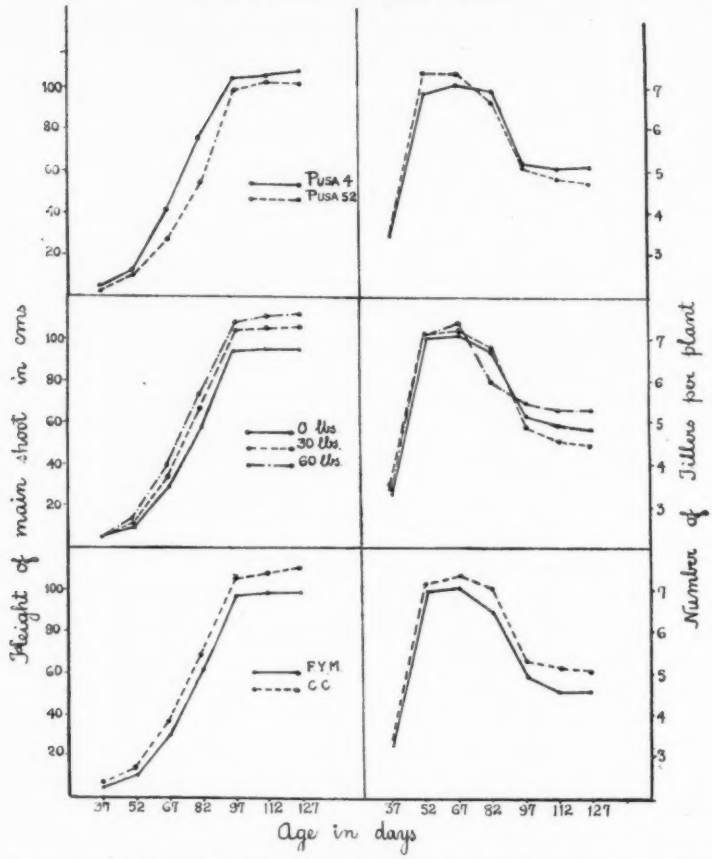

FIG. 2. Height and tillering in wheat in response to varieties, doses and forms of manure.

High yield under castor cake was, therefore, related to the better development of the plant under this manure. Greater height, and larger tillering, indicate that plants under this treatment exhibit greater vegetative vigour. This is undoubtedly traceable to higher percentage of nutritive ingredients other than nitrogen, made available to the crop under castor cake as compared to farm-yard manure. High nutrient concentration, greater vegetative growth and larger yield, therefore, appear interrelated.

B. Effect of levels of manure

So far as the level of manuring was concerned, the effects were found to be significant on height at all stages, on tillers at 37 and 112 days and on green leaves at 52 and 67 days. Ear characters, *e.g.*, number of spikelets,

number of grains, and yield of straw and grain were also significantly affected by doses. The higher the level of manuring the greater was the length of the main shoot, each successive increase being significant in effect. This was not so in tillering where only during the very early stages of 37 days, was the effect of 30 lb. dose positively significant; 60 lb. dose again improved tillering only at the last stage. On green leaves, the second increase from 30 to 60 lb. N improved their number at 52 days only. The first increase from 0–30 lb. on the other hand, showed positive increases in leaf number at 97 days only. Number of grains in the ear, absolute weight of grain and yield of grain and straw all improved under this 30 lb. treatment with manures. High yields under this level of manuring were associated with all round improvement in reproductive characters; the effect of higher doses, on the contrary, were prominent on vegetative characters. The heavier the nitrogen feeding the greater was the vigour induced in plants (Fig. 2). Quality of grain as judged by ash and nitrogen percentages was better at lower levels of 30 lb. than at 60 lb. feeding.

C. Responses of varieties

Of the two varieties, Pusa 4 proved to be better than Pusa 52 in some respects. This effect was observed on height at majority of stages, on tillers at 111 days and on green leaves at 52 days. Ear size and absolute weight of grain was also better in this case. Pusa 52, on the other hand, also showed improvement over Pusa 4 in other directions; thus tillering at 52 days, leaf number at 97 days, number of spikelets, number of grains per ear and yield of straw and grains were higher in Pusa 52 as compared with Pusa 4. An attempt was, therefore, made to determine the maximum or perultimate yield of the two varieties under the organic manure—castor cake, in accordance with the procedure laid down in the following equations[34]:—

$$A = \frac{y_2^2 - y_1 \cdot y_3}{2y_2 - y_1 - y_3} \tag{1}$$

$$c = \frac{\text{Log} \cdot (A - y_1) - \text{Log}\,(A - y_2)}{x_2 - x_1} \tag{2}$$

$$b = \frac{\text{Log}\ A - \text{Log}\,(A - y_1),}{c} \tag{3}$$

where A = perultimate yield or maximum possible yield under this manure, c = effect factor for the manure, b = basic nutritional level of soil in terms of lb. of the manure, y_1, y_2, y_3 = average yield under 0, 30 and 60 lb. N levels, and x_1, x_2, x_3 are the three doses of the manure.

Taking the average figures for total produce of straw and grain per acre under the three levels of 0, 30 and 60 lbs., calculations were made of A, *b* and *c*. The maximum yield of Pusa 4 under ideal conditions in response to castor cake has been estimated to be 67·4 mds. per acre in contrast to 59·4 mds. for Pusa 52. Pusa 4 therefore, is potentially capable of yielding higher (Table VII) than Pusa 52, though under the conditions prevailing at

TABLE VII

Total produce of straw and grain in mds. per acre under different doses of castor cake

Variety	Per-ultimate Yield A	Observed Yield			Expected Yield			Value of	
		0	30	60	0	30	60	c	b
Pusa 4 ..	67·4	40·4	49·7	55·8	..	49·98	55·77	0·0061	6·51
Pusa 54 ..	59·4	32·4	55·14	58·74	..	55·1	57·9	0·0267	12·85

the time of the experiment, it showed at an average 82·8 per cent. of its maximum yielding capacity. In contrast to this, it has been possible to exploit as much as 92·7 per cent. of the maximum yield of Pusa 52 under the same conditions in these soils. It is, therefore, one thing to exhibit an inherent potentiality for high yield and another thing to evince it in nature where it is often difficult to maintain an ideal environment with the manure only as the limiting factor in crop production. Pusa 52 is, therefore, likely to be more economical than Pusa 4. This is further corroborated by the figures for profit and loss worked out for the various treatments. The economics of manurial treatments [App. Tables I and II], show that manuring Pusa 52 with castor cake at 30 lb. N level gives the best return; treatments next in order of profit are (i) manuring Pusa 52 with castor cake at 60 lb. N level; (ii) manuring Pusa 4 with castor cake at 60 lb. N, each giving a net saving of Rs. 142, 116, and 105 respectively.

The expected values of yield of the two varieties at different levels of castor cake feeding ranging between 0 and 60 lb. N have been calculated after the equation:—

$$\text{Log } A - y = \text{Log } A - c\ (b + x), \qquad (4)$$

where the figures indicate the same factors as indicated in equation 1–3 given above. It is interesting to note that the expected and observed values are very nearly the same indicating that the crop has grown according to the

expected potentiality under 30 and 60 lbs. N levels of castor cake. The nature of the curve indicates that Pusa 52 is better than Pusa 4 at almost all levels of manuring shown in Fig. 3 (Table VIII). The potentiality of Pusa 52 for better growth thus is found to be decidedly superior to that of the other variety under the conditions prevailing at the time of the experiment.

TABLE VIII

Expected yield under various doses of castor cake

Dose in lb.	Expected yield	
	Pusa 52	Pusa 4
0	32·46	40·4
10	44·83	43·93
20	49·52	46·93
30	55·14	49·68
40	57·10	52·0
50	59·27	53·98
60	59·25	55·74
70	..	57·27

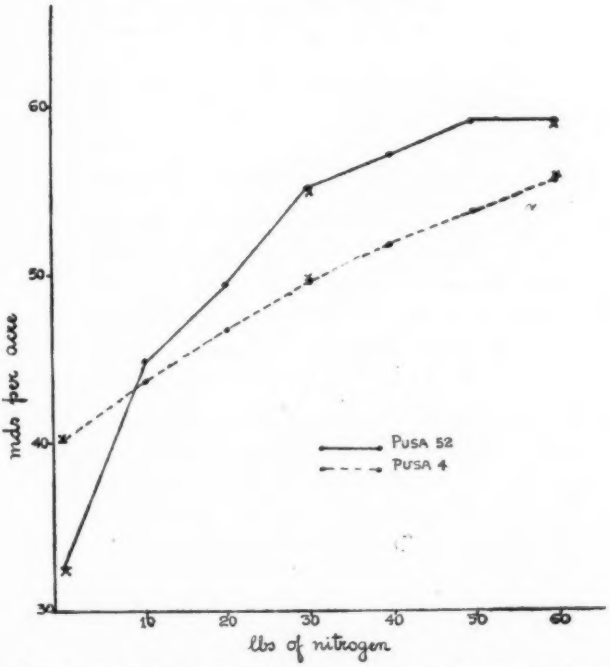

FIG. 3. Yield of straw and grain in two varieties of wheat. (•) Calculated, (x) observed yield.

Height/Tiller ratio.—Increases in height and tillering are the external manifestations of vegetative vigour of a variety. Balanced vegetative growth should, therefore, necessarily involve development of the plant in both these directions. Calculations of this ratio under different treatments, show that irrespective of the form, level or variety, the greater the age of the plant, the higher were the height/tiller ratios (Fig. 4). Castor cake-treated plants, as a general rule, showed a higher ratio than plants treated with farm-yard manure. Increase in dose of fertilisers, however, showed similar increases in ratio only upto 82 days in the life-cycle. Thereafter, addition of manures upto 30 lb. N increased the ratio while higher doses definitely lowered it. Among the varieties more or less similar response was noted. Thus

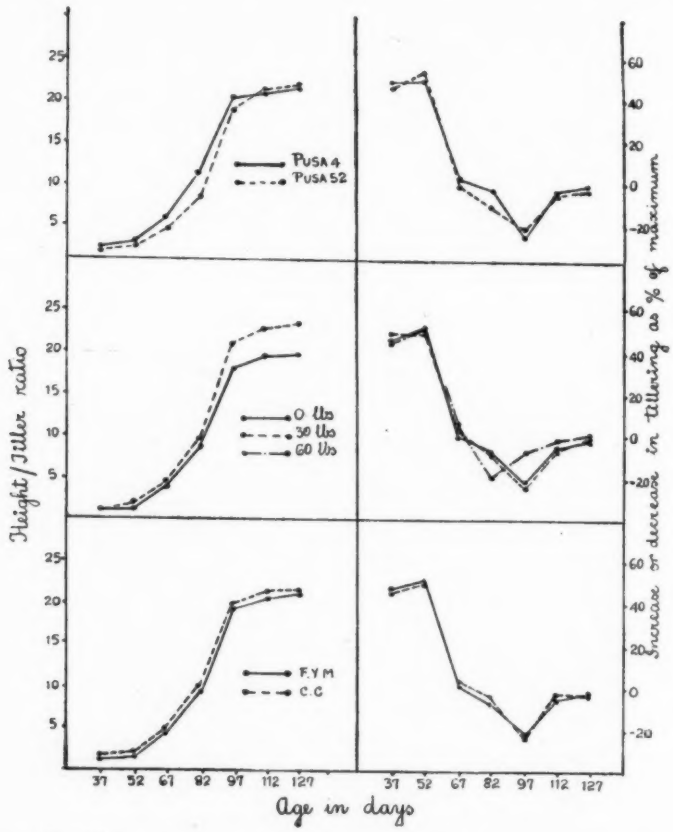

FIG. 4. Height/tiller ratio, and increase or decrease in tillering as percentage of maximum in response to variety, levels and forms of manure.

Pusa 4 showed higher ratio over Pusa 52 upto 97 days in the life-cycle; subsequently Pusa 52 tended to exhibit higher values.

A general tendency to exhibit a direct relation between this ratio and the capacity of the plant to yield under the influence of these manures appears evident. The higher the ratio the greater is the total produce of the crop. The average values taken at certain critical stages of development, such as the stage of maximum tillering (62 days) indicate that it is possible to visualise more or less accurately the order in which the yields of different plots would be located by merely arranging the height/tiller ratios at this stage in increasing order (Table IX). The grain and straw yield of the crop

TABLE IX

Average height/tiller ratio under different treatments and height grain and straw yield at harvest

Treatments	Height/tiller ratio*	Grain yield	Straw yield	Height Final stage
Average value for 0 lb. ..	4·05	7·0	19·57	97·6
,, ,, F.Y.M. ..	4·46	8·0	21·84	100·65
,, ,, 30 lb. ..	4·60	9·0	25·89	105·6
,, ,, Castor ..	4·99	9·83	27·13	108·9
,, ,, 60 lb. ..	5·4	10·75	27·73	111·4

* At the stage of highest tillering arranged in increasing order.

towards the end is found to vary in more or less the same order as the variations in the value of this ratio at the stage of maximum tillering. So is the case with the ultimate height of the crop. This ratio, therefore, provides an important tool to predict the ultimate effect that a fertiliser or a nutrient will have upon the final yield of crop. Significance of this ratio in judging the vegetative vigour shall be discussed elsewhere.

Critical period of vegetative development.—When the increases or decreases in number of tillers in between two successive intervals is expressed as percentage of the maximum, the contribution made by successive stages of growth towards the formation of tillers was worked out. Similarly, the increases in height at each successive time intervals were also expressed as percentage of the maximum height of the plants recorded in the life-cycle. The values thus obtained show that age factor was more important in bringing about variations in tillers at each stage than the factor for manuring. The

stage of maximum tiller development was located between 37–52 days when over 90 per cent. of tillers were formed (Fig. 4). Soon after a decline in tiller formation took place. It was significant to mark acute mortality of tillers between 82–97 days when as much as about 25 per cent. of tillers could not survive the stresses of the environment under which they grew. Subsequently a recovery in the sense of slackened mortality was noted in all manurial plots between 112–27 days in the life-cycle. There appeared to be two critical stages: (i) the stage of maximum tiller formation between 27–52 days and (ii) the stage of highest mortality between 82–97 days. The effects of forms, levels and varieties were less significant when compared to the changes brought about by age factor. What determined high rate of formation of tillers during early stages, and high rate of mortality during the second period remains yet to be worked out.

So far as increase in height is concerned, the data give evidence to the view that there was only one stage of critical development between 82–97 days when the increases in height took place to the extent of about 35 per cent. of the total recorded height (Fig. 5). The contributions made by subsequent stages was practically negligible. The life-cycle of the crop could thus be divided into two distinct halves: the first half between 37–97 days showed a period of increasing vegetative activity; the second between 97–156 days evinced a period of declining vegetative development of the crop. Variety Pusa 4 appeared to exhibit better vegetative development than Pusa 52 in the first half while the latter proved better than the first in the second part of the life-cycle (Figs. 4–5). Castor cake similarly proved more useful in the first half and less helpful in the second half. There was some tendency to exhibit increasing vegetative vigour with added doses of these manures only in the first half of the life-cycle. Here again, the age effects appeared to be more prominent than the manurial or varietal effects.

Relative growth rate.—The relative growth in height showed conclusively that changes induced by age were decidedly more important than those brought about by manures or doses. Except during the early stages, *viz.*, at the period of maximum growth rate (67 days) when castor cake was found to be better than farm-yard manure and when 60 lbs. level of manuring was better than 30 lbs. or no manuring, at other stages, the difference due to these factors was not at all sharp. At most of the stages between 82–112 days, variety Pusa 52 showed higher growth rate than Pusa 4. It is useful to indicate that with respect to forms of manure and levels of manuring, the higher the pitch of growth rate during early stages (67 days) the better was the ultimate performance of the crop specially in the matter of straw and grain yield (Fig. 5). In varietal comparisons, however, this relation between high yield

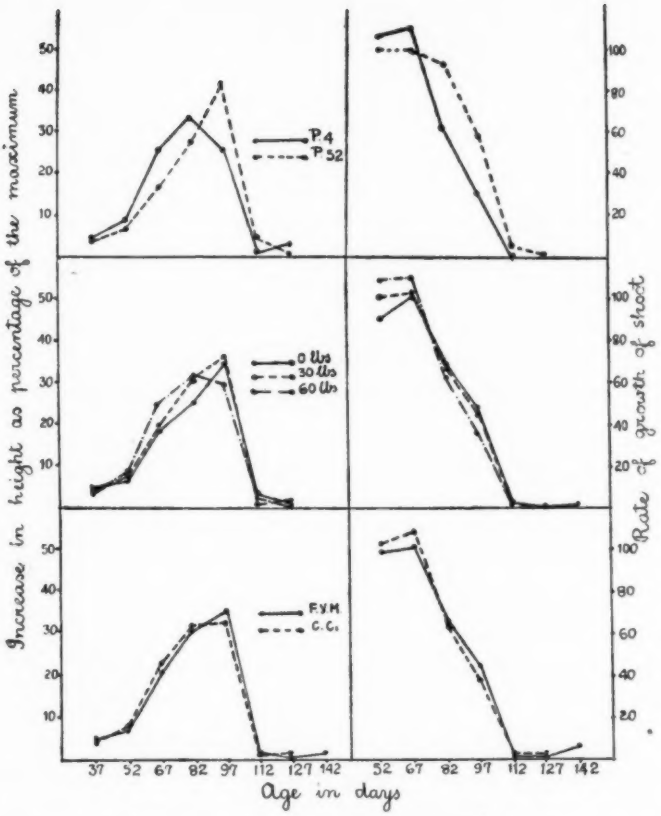

FIG. 5. Increase in height as percentage of maximum and rate of growth of shoot in response to varieties, levels and forms of manure.

and high growth rate was not evident. The better performance of Pusa 52 was due apparently to the increased rate of growth of this variety over that of Pusa 4 for a sufficiently long time in the life-cycle. The fundamental analysis of this problem of aging and senescence in relation to nutrient supply shall be undertaken in a later communication.

SUMMARY

The paper narrates the results of an experimental enquiry on the relative effects of castor cake and farm-yard manure when applied at three levels of 0, 30 and 60 lbs. N, upon the growth performance of two varieties of wheat, *viz.*, Pusa 4 and Pusa 52. The experiment was conducted under field condi-

tions, and the growth responses measured in terms of height of main shoot, number of tillers, green leaf number on main shoot, ear characteristics, straw and grain yield, ash and crude protein percentage of flour.

Of the two forms of manure, castor cake was undoubtedly better than farm-yard manure in inducing greater vegetative vigour, increasing yield, and improving height/tiller ratio and relative growth rate of plants. Age seemed to be having a more significant effect in altering the developmental physiology of the crop than the manures under consideration.

Different doses of manure brought about characteristic effects upon various developmental characters. High yields under lower levels of 30 lbs. were associated with an all round improvement in reproductive characters; higher doses, on the contrary, showed greater vegetative vigour in plants. Quality of grain was decidedly better at 30 lbs. manuring than at higher doses.

Mitscherlich yield tests showed that Pusa 52 though a potentially less efficient agrotype than Pusa 4, was under the soil and climate conditions of Banaras, a more useful variety. More than 90 per cent. of the maximum yielding capacity of this variety could be efficiently exploited under the conditions of experiment.

Manuring Pusa 52 with castor cake at 30 lbs. N level gave the maximum return, next in order of profit was manuring of Pusa 52 with 60 lbs. castor cake.

Age, forms of manure, and level of manuring brought about marked changes in height/tiller ratio. The higher the ratio at the stage of maximum tiller formation, the greater appeared to be the capacity of the plants to yield high. At other stages this relation was less evident.

Two critical periods of tiller formation, (i) the stage of maximum tillering between 37–52 days and (ii) the stage of maximum mortality between 82–97 days, were noted. Only one critical stage between 82–97 days was however noted for height. The life-cycle showed two distinct types of response—a period of high vegetative activity between 37–97, and a period of declining vegetative vigour between 97–156 days.

Irrespective of the treatments, growth rate was high during early stages and gradually fell down with advance in age. At the period of maximum growth rate, castor cake appeared to be better than farm-yard manure, and 60 lbs. dose better than 30 lbs. N level. Effects of these factors was not so significant at other periods.

Literature Cited

1. Bach, M. Landw .. *Versuchs. Stat.*, 1926, **104** (5) 6, 245–84.
2. Bartner, G. .. *Biol. Abs.*, 1935, **11**, 1576.
3. Barthel, C. .. *Fetschr. Landw*, 1926, 27–41.
4. Bartholomew, R. P. .. *Jour. Amer. Soc. Agron.*, 1928, **20** (1), 55–81.
5. Batham, H. N. .. *Soil Science*, 1927, **24** (3), 187, 203.
6. Blair, A. W. and Prince, A. L. .. *Ibid.*, 1928, **25** (4), 281–87.
7. Bretigniere .. *Rend Acad. Agri. France*, 1929.
8. Daji, J. A. .. *Jour. Amer. Soc. Agron.*, 1934, **26** (6), 461–75.
9. Dumond, R. .. *Bull. Econ. Indochina*, 1930, 33 (4B), 9–31.
10. Ivanovskaia, T. L. .. *Biol. Abs.*, 1933, **7** (9), 21853.
11. ———— .. *Rep. Transcaucasian Cotton Res. Inst. Tiflis*, 1932, 221–46.
12. Holtz, H. F. and Vandecaneye, S. C. *Soil Sci.*, 1938, **45** (2), 142–63.
13. Kielpinski .. *Roczinki Nauk Rolniczynch Lesnych*, 1933, **30** (2), 281–83.
14. Lemmermann, O. W. .. *Jessen, H. Engel Zitser, Wisserisch*, 1930.
15. Lipman, J. G. and Blair, A. W. .. *Proc. First International Congress Washington, D. C.*, 1928, **3**, 454–64.
16. ———— .. *Soil Sci.*, 1928, **26** (i), 1-25.
17. Loehwing, W. F. .. *Proc. Iowa. Acad. Sci.*, 1927, **34**, 149–52.
18. Mcallister, R. E. .. *Sci. Agric. (Ottawa)*, 1934, **14** (5), 249–56.
19. McGeorge, W. T. .. *Journ. Amer. Soc. Agron.*, 1934, **26** (7), 575–79.
20. Mckibbin, R. R. .. *Biol. Abs.*, 1934, 8 (5).
21. Odland, T. E. and Smith, J. B. *Journ. Amer. Soc. Agron.*, 1933, **25** (9), 612–18.
22. Raden, W. R. .. *Amer. Fert.*, 1934, 80 (10), 5722.
23. Richardson, A. E. V. and Gurney, H. C. .. *Emp. Journ. Exp. Agri.*, 1933, **1**, 193–205, 325–32.
24. Rolet, A. .. *Agri. Prat Pays, Chands*, 1931, 1931(11), 354–64 ; **12**, 430–44.
25. Sackett, W., Ida, G. A. K.. Ferguson, W. & Ward, J. C. *Biol. Abs.*, 1929, **3**.
26. Salter, F. J. .. *Jour. Amer. Soc. Agron.*, 1927, **19** (5), 397–409.
27. Salter, R. M. and Green, T. C. *Ibid.*, 1938, **25** (9), 622–30.
28. Sievers, F. J. and Holtz, H. F. *Proc. First Int. Cong. Soil Sci., Washington, D. C.*, 1927–28, 3423–436.
29. ———— .. *Washington Agri. Expt. Sta. Bull.*, 1926, **43**, 206.
30. Singh, B. N., *et al.* .. *Progress Report of Researches on Physiology of Wheat and Cane, I. C. A. R.*, 1938–40.
31. Stoklasa, J. .. *Trop. Agri.*, 1927, 68 (3), 156–63.
32. Waksman, S. and Florence, G. T. *Soil Science*, 1928, 26 (2), 155, 171.
33. White, J. W. .. " Crop Yields, etc.," *Journ. Amer. Soc. Agron.*, 1935, **23** (6).
34. Wilcox, O. W. .. *Principles of Agrobiology.*

APPENDIX

TABLE I

Average cost of cultivation of wheat per acre*

Particulars of expenses		Amount in Rs.
1 Land rent	..	20 0 0
2 Seed	..	12 0 0
3 Nine ploughings and sowing	..	24 12 0
4 Irrigation	..	42 0 0
5 Harvesting	..	7 0 0
6 Threshing and winnowing	..	14 0 0
7 Machinery depreciation charges	..	8 0 0
8 Supervision and general watch	..	13 0 0
Total	..	140 12 0

* Calculated at rates prevalent during 1943–44.

APPENDIX

TABLE II

Cost of production and return per acre of differently treated plots

Treatments	Cost of production				Gross income				Profit			
		Rs.	A	P.		Rs.	A.	P.		Rs.	A.	P.
1 Variety Pusa 52 F.Y.M. at 30 lb. N	Cultivation ..	140	12	0	Straw	67	9	0	Income	227	9	0
	Manuring ..	17	0	0	Grain	160	0	0	Expenses	160	4	0
	Distribution of manure	2	8	0								
	Total ..	160	4	0	Total	227	9	0	Profit	67	5	0
2 Variety Pusa 4 C.C. at 30 lb. N	Cultivation ..	140	12	0	Straw	83	7	0	Income	230	7	0
	Manure ..	31	12	0	Grain	147	0	0	Expenses	175	0	0
	Distribution of manure	2	8	0								
	Total ..	175	0	0	Total	230	7	0	Profit	55	7	0
3 Variety Pusa 52 C.C. Control	Cultivation ..	140	12	0	Straw	69	0	0	Income	182	6	0
	Manure and manuring		..		Grain	113	6	0	Expenses	140	12	0
	Total ..	140	12	0	Total	182	0	0	Profit	41	10	0
4 Variety Pusa 4 C.C. at 60 lb.	Cultivation ..	140	12	0	Straw	120	0	0	Income	312	0	0
	Manure ..	63	8	0	Grain	192	0	0	Expenses	206	12	0
	Distribution of manure	2	8	0								
	Total ..	206	12	0	Total	312	0	0	Profit	105	4	0
5 Variety Pusa 52 F.Y.M. at 60 lb. N	Cultivation ..	140	12	0	Straw	101	8	0	Income	258	14	0
	Manure ..	34	0	0	Grain	157	6	0	Expenses	177	4	0
	Distribution of manure	2	8	0								
	Total ..	177	4	0	Total	258	14	0	Profit	81	10	0

TABLE II—(*Contd.*)

Treatments	Cost of production		Gross income		Profit	
6 Variety Pusa 4 C.C. Control	Cultivation .. 140 12 0 Manures and manuring ..		Straw 92 12 0 Grain 108 8 0		Income 201 4 0 Expenses 140 12 0	
	Total .. 140 12 0		Total 201 4 0		Profit 60 8 0	
7 Variety Pusa 52 F.Y.M. Control	Cultivation .. 140 12 0 Manures and manuring ..		Straw 82 4 0 Grain 122 12 0		Income 205 0 0 Expenses 140 12 0	
	Total .. 140 12 0		Total 205 0 0		Profit 65 4 0	
8 Variety Pusa 4 F.Y.M. at 60 lb. N	Cultivation .. 140 12 0 Manures .. 33 14 0 Distribution of maure 2 8 0		Straw 93 12 0 Grain 145 12 0		Income 239 8 0 Expenses 177 2 0	
	Total .. 177 2 0		Total 239 8 0		Profit 62 6 0	
9 Variety Pusa 52 C.C. at 60 lb. N	Cultivation .. 140 12 0 Manures .. 31 12 0 Distribution of manure 2 8 0		Straw 125 7 0 Grain 192 0 0		Income 317 7 0 Expenses 175 0 3	
	Total .. 175 0 0		Total 317 7 0		Profit 142 7 0	
10 Variety Pusa 4 F.Y.M. Control	Cultivation .. 140 12 0 Manures and manuring ..		Straw 69 12 0 Grain 97 0 0		Income 166 12 0 Expenses 140 12 0	
	Total .. 140 12 0		Total 166 12 0		Profit 26 0 0	
11 Variety Pusa 52 C.C. at 60 lb. N	Cultivation .. 140 12 0 Manures .. 63 8 0 Distribution of manure 2 8 0		Straw 131 0 0 Grain 192 0 0		Income 323 0 0 Expenses 206 12 0	
	Total .. 206 12 0		Total 323 0 0		Profit 116 4 0	
12 Variety Pusa 4 F.Y.M. 30 lbs. N	Cultivation .. 140 12 0 Manures .. 17 0 0 Distribution of manures 2 8 0		Straw 77 7 0 Grain 80 12 0		Income 158 3 0 Expenses 160 4 0	
	Total .. 160 4 0		Total 158 3 0		Loss 2 1 0	

OBSERVATIONS ON THE CHEMISTRY OF THE WATERS OF THE BAY OF BENGAL OFF MADRAS CITY DURING 1948-1949

By R. Jayaraman, M.A.

(*Central Marine Fisheries Research Station, Mandapam Camp*)

Received July 11, 1950

(Communicated by Dr. H. Srinivasa Rao, F.A.SC.)

Introductory

The study of the fundamental problem of the productivity of the sea involves a knowledge of the composition of the sea water, particularly with reference to those inorganic compounds which form a valuable link in the food chain of life in the sea. The most important among these are the compounds of phosphorus, nitrogen and silicon. Studies on the distribution and seasonal variation of these compounds, commonly referred to as 'nutrients', form an essential preliminary to a more detailed biological investigation of the sea. Much work on these lines has been done by several workers abroad (*vide* Harvey, 1945), but their investigations were mostly confined to the waters of the temperate and colder regions. We do not have much information regarding the chemical conditions of the waters around the Indian coasts. Except for the investigations of Sewell (1928–29), Thompson and Gilson (1937) and Bal *et al.* (1946), little systematic work appears to have been done in our waters.

The present work was undertaken as a preliminary survey of the waters of the Bay of Bengal in the vicinity of Madras. Studies were made on the distribution and seasonal variations in concentration of phosphates, nitrates, nitrites and silicates during a period of 17 months—March 1948 to the end of July 1949. Simultaneous observations were also made on the variations in the salinity and dissolved oxygen of sea water.

Experimental

Collection of Samples

Samples of sea water for analyses were collected from the surface twice a week in the early hours of the morning at a distance of about 3 miles offshore near the Marina Beach. Glass-stoppered Pyrex bottles, 600-1,000 ml. capacity were employed for samples intended for general analyses and special 200 ml. bottles for dissolved oxygen determinations.

Analytical Procedure

Phosphates.—The estimation of phosphates was done by the ceruleo-molybdate method of Denigés modified by Atkins (1923). Nessler cylinders were used and the comparison of colour was made in daylight. The values given have not been corrected for 'salt error'.

Nitrates.—Harvey's Reduced Strychnine method (1926, 1929) was employed with the following modifications: The time of reaction was reduced to 1 hour in the light of the suggestions by Riddell (1936) and Rochford (1946). In the place of the original undiluted reagent of Harvey's, one diluted in the proportion of 1 to 3 with nitrate-free sulphuric acid was made use of. The nitrate-free acid for the purpose was prepared by the method of Ramachandran (1948). The diluted reagent proved to be much more sensitive than the original one. The colour developed was estimated in a Hellige-Duboscq Colorimeter.

Nitrites.—The nitrites were estimated by the method of Griess-Ilosvay worked out in detail for sea water by Orr (1926), and modified later by Robinson and Thompson (1948).

Silicates.—The method of Diénert and Wandenbulcke (1923) as adopted by Atkins (1926) was used. The standard for colour comparison was a

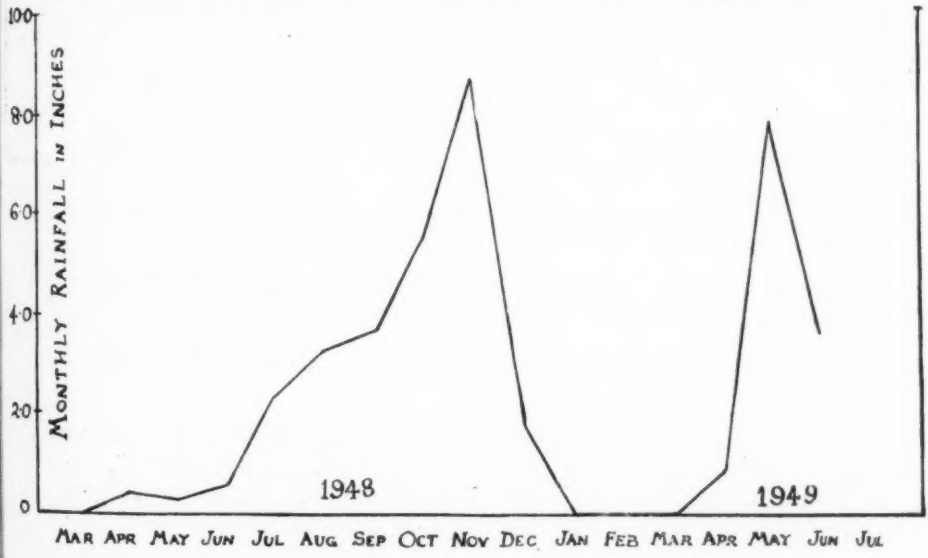

FIG. 1. Showing the monthly rainfall in Madras City during the period 1948–49) From the Reports of the Regional Meteorological Centre, Madras).

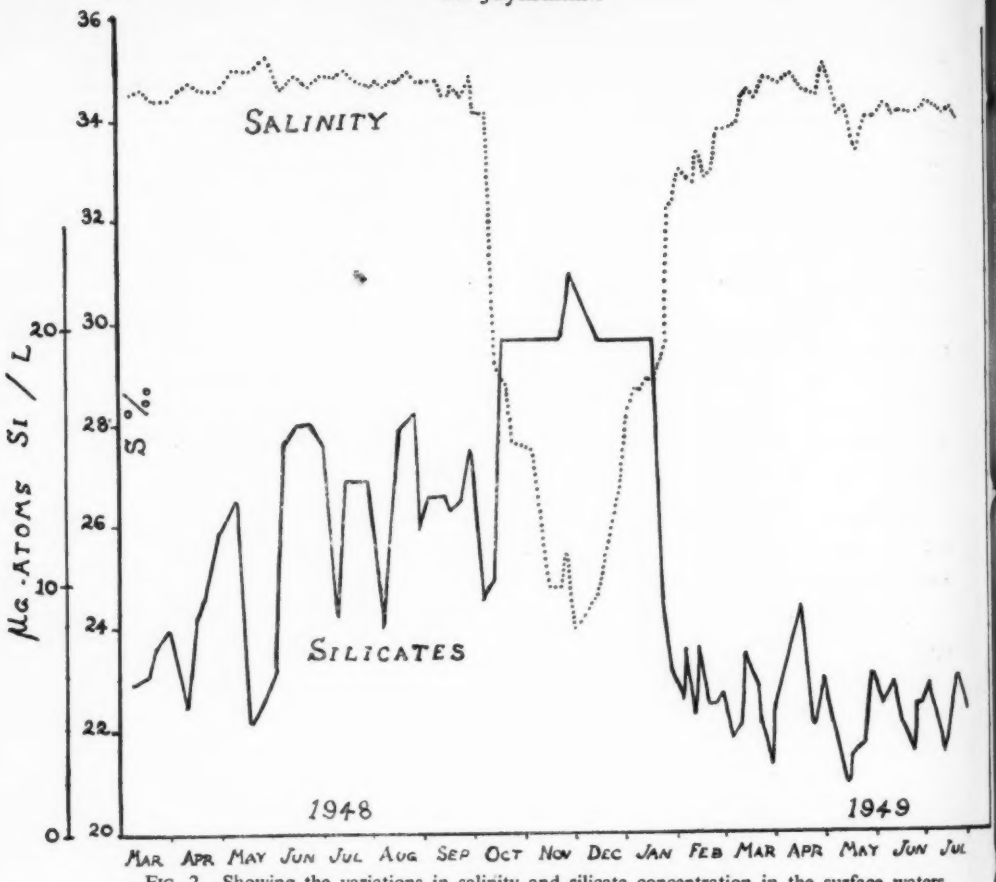

FIG. 2. Showing the variations in salinity and silicate concentration in the surface waters of the Bay of Bengal during the period 1948–49.

solution of potassium chromate buffered with borax as described by Thompson and Houlton (1933).

Oxygen.—The dissolved oxygen was estimated by the Winkler's method (Harvey, 1928).

Salinity.—The salinity determinations were done by the titration procedure of Oxner and Knudsen as described in detail by Harvey (1945). In the early part of the investigation the standard used was a solution of pure sodium chloride containing 32·34 gm. of NaCl per litre and later Normal Sea Water of chlorinity, 19·381‰, supplied by the Hydrographical Laboratories, Copenhagen, was used.

Results

The results obtained are shown graphically in Figs. 2, 3 and 4. The concentrations of the nutrients are expressed as microgram-atoms per litre following the international convention, and the oxygen content as ml./L. In Fig. 1 is represented the monthly rainfall for Madras for the period 1948–49.

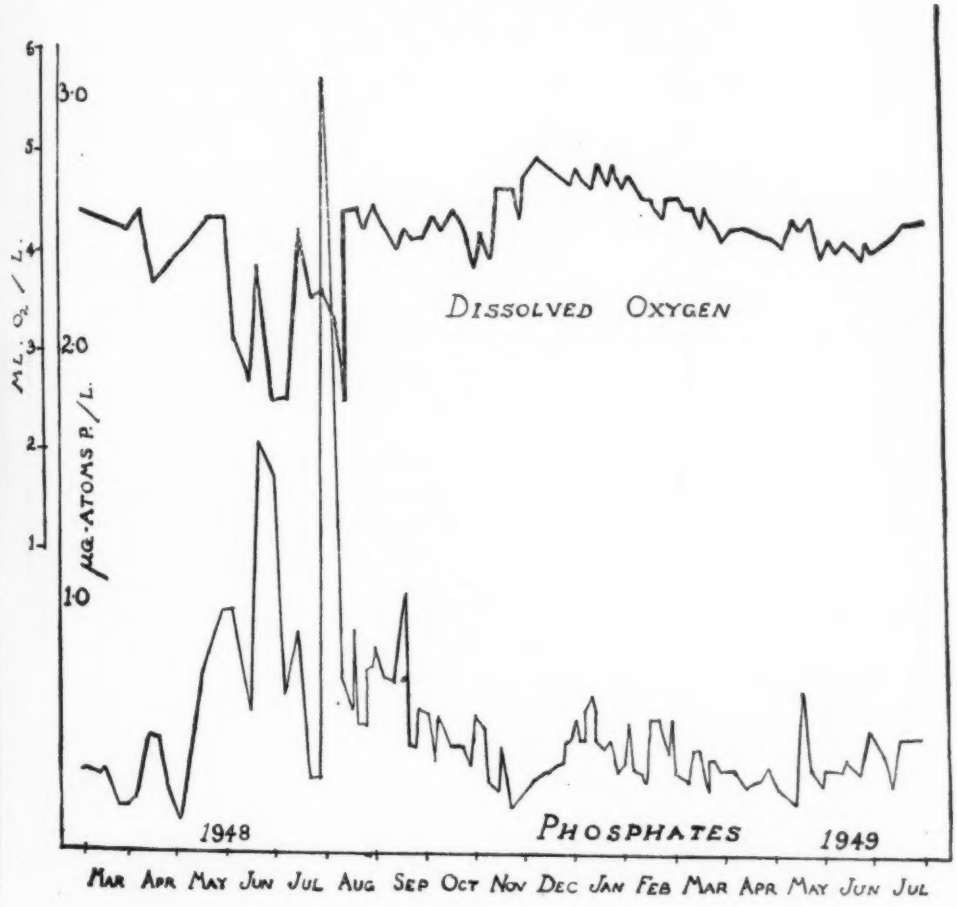

FIG. 3. Showing the distribution of Phosphates and dissolved Oxygen in the surface waters of the Bay of Bengal during 1948–49.

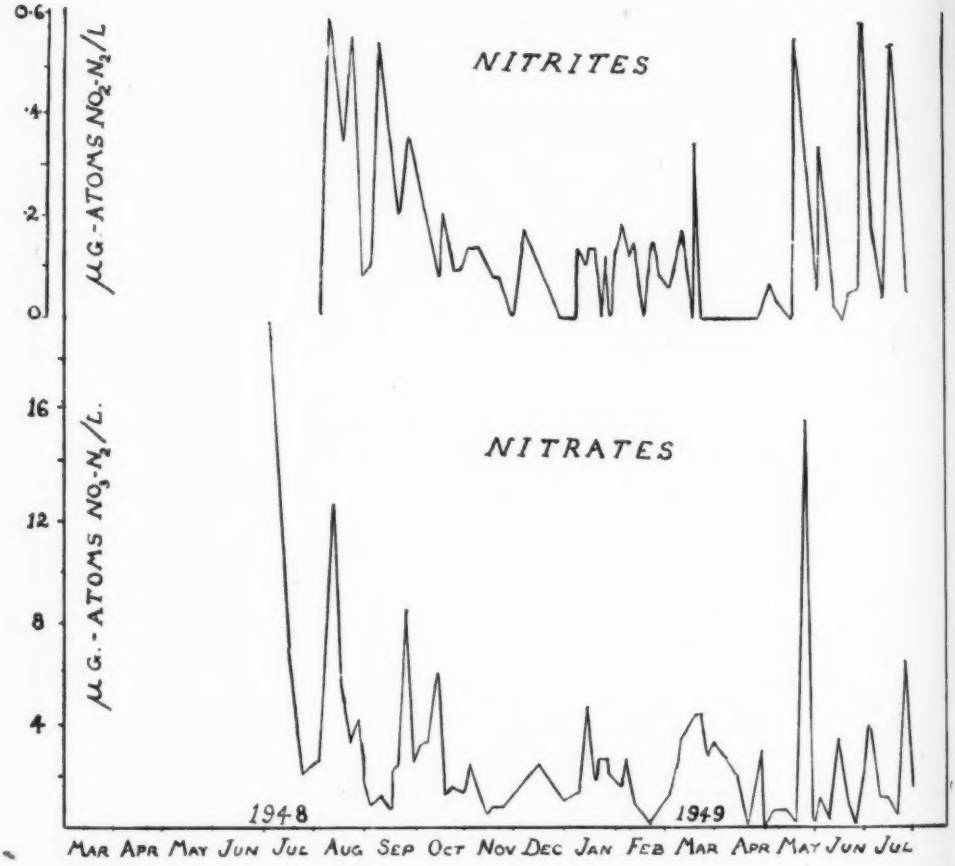

FIG. 4. Showing the nitrate and nitrite content of the surface waters of the Bay of Bengal during 1948–49.

DISCUSSION

The data presented here show several interesting features. It would be proper to consider each of the constituents individually and indicate its relationship to the others.

Salinity.—The importance of salinity as an index of water movements is well known and data indicating the variations in salinity are useful in explaining the causes underlying the fluctuations in the nutrients and other constituents. The seasonal variations in salinity are shown in Fig. 2. It is seen that the salinity has been constant from March to the end of September 1948. By the middle of October, a downward trend is indicated and

is continued till the end of December. A similar lowering in salinity in the surface waters of the Bay of Bengal at this part of the year has been recorded by Sewell (1928–29). It is seen from his charts that a strong southerly current runs along the east coast at this part of the year, carrying southwards " the low density waters from the mouth of the Godavari ", and the lowering in salinity of the surface waters of the Bay may, therefore, be attributed to this influx of fresh water. A comparison of the rainfall data for Madras (Fig. 1) with the salinity shows that the lowering in salinity occurs at the period when the rainfall is at its maximum. It may, however, be pointed out the local rainfall alone could not have been responsible for the very low salinity noticed during the period; for it is to be observed that there is no appreciable lowering in salinity corresponding to the second peak —that is in the month of May, 1949—in the rainfall graph. Cooper (1933), too, has observed similar lowering in the salinity of the surface waters of the English Channel during certain months of the year, and he explaines it as being due to the pouring in of large volumes of fresh water from the rivers following heavy rains in the preceding months.

Phosphates.—The phosphate content of the surface waters of the Bay of Bengal is shown in Fig. 3. The highest concentrations are to be found in the months, May to September and the lowest from October to March. The seasonal fluctuations appear to be as marked as shown by other workers for the northern waters. Harvey (1928, 1930) has reported that phosphates in the surface waters of the English Channel show maximum concentrations during October to March and minimum during the rest of the year. It is of interest that lowest concentrations of phosphates are found in our waters from October to March. Dakin and Colefax (1935) have reported similar seasonal fluctuations in the southern hemisphere, but according to them, the average during the summer months is not appreciably less than during the winter months.

The seasonal decrease of phosphates in water due to the photosynthetic activity of the phytoplankton is well known from published data of different workers in many areas of the world. The values for dissolved oxygen is a fairly good index of the activity of the phytoplankton and a comparison of the graph (Fig. 3 top) representing the dissolved oxygen content with that representing phosphates (Fig. 3 bottom) shows that in the period in which the phosphates were maximum, the dissolved oxygen was at its lowest and *vice versa.*

Nitrates and Nitrites.—The distribution of nitrates and nitrites is shown in Fig. 4. The determination of these constituents could not be commenced earlier than July-August 1948.

An examination of the graph showing the distribution of nitrates reveals a close similarity to that of phosphates. The period of maximum concentration corresponds to that of phosphates. It is, however, seen that there is no constancy in the relative concentration of these two constituents, in other words, the N/P ratio appears to be variable unlike what has been reported for oceanic waters. Redfield (1934) was the first to point out the quantitative relationship between nitrates and phosphates, and it has been further substantiated by Cooper (1937). The ratio of 15:1 for N:P as arrived at by Cooper was, however, lower than that obtained by Redfield, but the values obtained by later workers were more in conformity with the observations of Cooper. It may be of interest to point out that in our waters the ratio of nitrate to phosphate has been consistently very low. It has been suggested by Riley (1941) from his investigations on the waters of the Georges Bank that such low values are characteristic of shallow regions, because the rapid return to the surface of the nutrients regenerated at the bottom leads to a preponderance of the more quickly regenerated phosphate. Moreover, as stated by Redfield (1934), the proximity of the land, the inflow of rivers and the peculiarities of the local flora and fauna may have a marked influence on the nitrate-phosphate relationship in our waters.

The nitrites show variations similar to the nitrates, the two curves being almost parallel to each other.

Silicates.—The silicate content of the surface waters during the period under study is represented in Fig. 2 along with the salinity. The distribution of this constituent appears to be influenced to a great extent by water movements. The southerly drift of fresh water which causes lowering of the surface salinity during the months October to December, brings about a rise in the concentration of silicates. It is well known that river waters are rich in silicates, and this increase in the silicate content could, therefore, be attributed to the influx of the river waters.

Summary

Studies were made on the distribution and seasonal variation of salinity, dissolved oxygen and nutrients in the surface waters of the Bay of Bengal in the vicinity of Madras during 1948–49. The various constituents show marked seasonal fluctuations. The variations of phosphates and nitrates during the period appear to be more regular than those of silicates. The influx of fresh water at a certain season during the year is offered as an explanation for this phenomenon.

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to Dr. N. K. Panikkar for his valuable suggestions and to Dr. H. Srinivasa Rao, Chief Research Officer, Central Marine Fisheries Research Station, Mandapam Camp, for his kind permission to publish this paper.

REFERENCES

1. Atkins, W. R. G. .. *J. Mar. Biol. Assn. U.K.*, 1923, **13**, 119.
2. ——— and Wilson, E. .. *Biochem. Journ.*, 1926, **20**, 1223.
3. Bal, D. V., Pradhan, L. B. and Gupte, K. G. *Proc. Ind. Acad. Sci.*, 1946, **24B**, (2), 60.
4. Cooper, L. H. N. .. *J. Mar. Biol. Assn. U.K.*, 1933, **18**, 677.
5. ——————— .. *Ibid.*, 1937, **22**, 177.
6. Dakin, W. J. and Colefax, A. N. *Proc. Linn. Soc. N.S.W.*, 1935, **60**, 303.
7. Dièkert, F. and Wandenbulcke, P. *C. R. Acad. Sc., Paris*, 1923, **176**, 1478.
8. Harvey, H. W. .. *J. Mar. Biol. Assn. U.K.*, 1926, **14**, 71.
9. ——————— .. *Rapp. et Proc. Verb. Cons. perm. int. Explor. de la Mer.*, 1929, **53**, 68.
10. ——————— .. *Biological Chemistry and Physics of Sea Water*, Cambridge Univ. Press, 1928.
11. ——————— .. *Recent Advances in Chemistry and Biology of Sea Water*, Cambridge Univ. Press, 1945.
12. Orr, A. P. .. *J. Mar. Biol. Assn. U.K.*, 1926, **14**, 55.
13. Ramachandran, B. V. .. *Nature*, London, 1948, **162**, 458.
14. Redfield, A. C. .. *James Johnstone Memorial Volume*, Univ. of Liverpool Press, 1934.
15. Riddell, W. A. .. *J. Biol. Canada*, 1936, **Bd. 2**, 1.
16. Riley, G. A. .. *Bull. Bingh. Oceanogr. Colln.*, 1941, **7**, Art. 4.
17. Rochford, D. .. *Austr. C. S. I. R. Bull.*, 1946, 220.
18. Robinson, R. J. and Thompson, T. G. *J. Mar. Res.*, 1948, **7**, 42.
19. Sewell, R. B. S. .. *Mem. Asiat. Soc. Bengal*, 1928–29, **9**, No. 5.
20. Thompson, E. F. and Gilson, H. C. *Sci. Rep. John Murray Expedn.* (1933–34), 1933, **2**, No. 2, 15–76.
21. Thompson, T. G. and Houlton, H. G. *Ind. Eng. Chem.*, Anal. Edn., 1933, **5**, 417.

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ERRATA

Vol. XXX, No. 5, Sec. B, 1949, page 271, first para, lines 2–3—
"100–20" *should read* "100–120".

Vol. XXXII, No. 1, Sec. B, 1950, page 17, last para, line 3—
"Adrenaline (1 in 10,000)" *should read* "(1 in 100,000)".

Vol, XXXII, No. 3, Sec. B, 1950—

Page 117, Table VI, Column 4, the last two lines
should read as $B > C > D = E > G = H = F = A$.

Page 120, line 2, Plate XX *should read as* Plate I.

Page 125, Explanation to Plate XX
should read as Explanation to Plate I.

OBSERVATIONS ON THE LIFE-HISTORY OF THE INDIAN SHAD, *HILSA ILISHA* (HAMILTON)[1]

By S. Jones and P. M. G. Menon

(*Central Inland Fisheries Research Station, Barrackpore*)

Received April 26, 1950

(Communicated by Prof. L. Rama Rau)

Introduction

The life-history of the Indian Shad, *Hilsa ilisha* (Hamilton), began to receive the attention of fishery workers in India at a comparatively early date. Investigations were first taken up in Madras and soon after in Bengal, where attempts were also made at artificial fertilization so as to liberate the fry into the rivers to supplement the natural stocks as in the case of the American Shad, *Alosa sapidissima*. Wilson (1909) succeeded in artificially fertilizing the eggs of *Hilsa* and in liberating the hatchlings into the Coleroon River, South India. Since then, the Madras Fisheries Department have followed his lead and liberated 10 million fry in 1916 (Raj, 1917). Similar attempts, made in Bengal, did not meet with complete success (Southwell and Prashad, 1918). In Madras, however, the collection and hatching of *Hilsa* continued as a routine (Devanesan, 1939) resulting in valuable data being gathered on the probable breeding grounds,[2] but the actual spawning grounds remained elusive as no laid eggs or prolarvæ were ever collected from the rivers.

Hora (1938) made a significant contribution towards the location of the breeding grounds when he discovered the postlarvæ and young stages of *Hilsa* in the settling tanks and the filter beds of the Water Works of the Calcutta Corporation at Pulta, about 16 miles north of Calcutta. As the Hooghly water at Pulta was pumped into the Water Works, he inferred that the fish breeds in the river in the vicinity of Pulta and that the Hooghly as a whole serves as one of the spawning grounds of the fish. As a result of their continued observations Hora and Nair (1940 *a*) concluded that the fish breeds in the Hooghly in all the months of the year except November to March; and attributed their failure to collect the eggs or the larvæ

[1] Published with the permission of the Chief Research Officer, Central Inland Fisheries Research Station, Barrackpore.

[2] Madras Fisheries Department Administration Reports for 1930–31, p. 32 (1932); 1933–34, p. 41 (1934); 1934–35, p. 38 (1936); 1935–36, pp. 37–38 (1937); and 1936–37, p. 34 (1938).

from the river to the unsuitability of the appliances available to them, presuming that they were to be found " near the bottom in deeper waters in the middle of the river".

[3]Apart from a brief statement by Southwell and Prashad (1918) on the fertilization of *Hilsa* egg, the only published record of developmental stages of the *Hilsa* is by Nair (1939), who described postlarvæ and young stages of the fish obtained from the Pulta Water Works. In Nair's account, however, there is some confusion in the identity of the stages from 14 to 18 mm. described and figured by him. His description and the figures of *Hilsa* (from 14 mm. to 18 mm.) do not tally with the diagramatic representation of the relative positions of the dorsal fin and anus to the vertebræ (Nair, *op. cit.*, Plate V). In no stage in the development of the fish, have we found the anus or the anal fin situated in a position below the dorsal fin (Nair, *op. cit.*, pp. 410–12).

Jenkins' (1938) anticipation of the discovery of the eggs and early larval stages from the rivers has been amply justified by our collection of such laid eggs and larvæ both from the Hooghly near Pulta, and from the mouth of the Daya River (Chilka Lake). Some of spawning grounds of the fish have, therefore, been definitely located for the first time (Jones and Menon, 1950 *b*) and the development worked out in fair detail.

The following account is based on the observations carried out in the Hooghly River along a stretch of about 3 miles near Barrackpore, in the Mahanadi River at Kujang, in the Bay of Bengal at Jaunput on the Contai Coast, and in the Chilka Lake at the mouth of the Daya River.

Materials and Method

(*a*) *Collection of larval stages from the Hooghly.*—Collections of Clupied prolarvæ and postlarvæ were made from the Hooghly in the neighbourhood of Pulta from September, 1949 onwards. They consisted of 2 different types of larvæ. One was that of the Gangetic Anchovy, *Setipinna phasa* (whose eggs were also obtained), and the other was that of the *Hilsa* (Jones and Menon, 1950). During the same time postlarvæ and juvenile *Hilsa* were present in large numbers in the ' Bhin-jals' (conical bag nets) operating in the river. The *Hilsa* larvæ showed a remarkable peak occurrence during the second week of November, when yolked larvæ and postlarvae were collected in exceedingly large numbers in both surface and sub-surface hauls; but the eggs of *Hilsa* did not occur in these collections. In the collections made during the following December and January, the yolked larvæ

[3] See the addendum at the end of the paper.

of *Hilsa* were absent, but *Setipinna phasa* was breeding as was evident from the regular occurrence of its eggs.[4]

(*b*) *Collection of* Hilsa *eggs from the Hooghly.*—Towards the end of January, 1950, the presence of stray adult *Hilsa* in the river was observed. On the 2nd of February, 1950, we succeeded for the first time in collecting the eggs of *Hilsa* along with larval stages in our plankton net hauls and thereafter fairly regularly except for a few days (from 12th to 16th of February) when a cold spell prevailed.

(*c*) *Collection of the eggs and the larvæ from the River Daya.—Hilsa* eggs and larvæ were collected by one of us (S. J.) from the mouth of the River Daya—one of the deltaic branches of the Mahanadi—on the 16th and 17th of September, 1949, near the villages of Jagadal and Garasaguda respectively during one of the investigational tours to these places. This stretch of the river is situated close to its point of discharge into the Chilka Lake. The exact identity of these eggs was determined only after working out the development of the *Hilsa* from the collections in the Hooghly.

(*d*) *Method.*—Collections of eggs and larvæ were made from a 3-mile stretch of the Hooghly below the Pulta Water Works up to the Titaghur Paper Mills. A bolting silk net (course) was tied to a country boat by means of a rope and towed against the current. Hauls were made at different depths along the river generally up to a distance of 50 to 75 yards away from the shore and occasionally across the river. The plankton net was usually lowered to the desired depth with the aid of a punting pole. The flow of the current served to keep the net straight and its mouth open. Eggs and larvæ were present in larger numbers nearer the banks than in the middle of the river where the current was stronger. We collected both larvæ and eggs from shallow regions of about 18 inches depth. The post-larvæ were obtained in greater numbers in the hauls nearer to the surface than to the bottom, whereas the eggs were present usually in the sub-surface and bottom collections. Due to the upwelling of the water caused by the swirl of the current, the eggs were sometimes obtained in the surface hauls also, even though they are demersal in still water.

The embryonic and larval stages described and figured up to the 5th day are from the laid eggs collected from the Hooghly which were reared in the laboratory, and the later stages from the collections made from the river. For whole mounts and myotome counts, they were stained in

[4] A detailed account of the life-history and bionomics of *Setipinna phasa* (Ham.) is being published elsewhere.

Eosine (aqueous), and for observing the skeletal parts, they were stained in Alizarin Red.

All the diagrams were executed by the junior author (P.M.G.M.) under the supervision of the senior author.

Spawning Habits

There is a fair amount of literature on the spawning habits of *Hilsa*. The ascent of *Hilsa*, first recorded by Hamilton (1822), far above the tidal limits of flooded rivers, is now well known. The available records indicate that the main breeding season is during the monsoons (Day, 1878, Hora and Nair, 1940 *a* and Chacko and Ganapati, 1949), from June to September and October to December, and in the Hooghly River, where detailed investigations were carried out (Hora and Nair, *op. cit.*) the breeding extends almost throughout the year with a peak period during the rainy season. Our own observations in the Hooghly show that the breeding is very restricted, if not at a stand still, during the winter months of December and January, for, in the routine plankton hauls, the prolarvæ and the postlarvæ were scanty or absent. However, it is quite possible that the fish may even then be breeding lower down in the estuary where the temperature is higher. Detailed investigations in the Chilka Lake indicate that the fish there breed mainly during the monsoon months (July to October) when the Daya River and its connected branches are in floods. As stated by Day (1878), the rapidity of the current is probably an important factor amongst others in stimulating the spawning migration.

The ratio of males to females is generally constant with a preponderance of males at the commencement of the breeding season and of females during the period of spawning. In several instances shoals happened to consist of individuals of the same sex, male or female. Chacko and Ganapati (*op. cit.*) observed that in the Godavari both males and females "move together in shoals" in the proportion of 1 : 9 respectively; but such high disparity in the relative proportions of the sexes has not been noticed either in the Hooghly or in the Chilka Lake.

The female fish usually attains a larger size than the male. Chacko and Ganapati (*op. cit.*) record a weight of 5½ lb. for a female specimen 23·5 inches long, and about 1¼ million eggs in a specimen 2 lb. in weight. Oozing males about 9 inches long have been taken by us in the Hooghly River and Chilka Lake.

Raj (1941) recorded that the eggs are laid in the evening. The earliest stage we have collected is the one with the blastoderm formed as a cap of

cells. Such stages were obtained from the Hooghly in February 1950 between 2 P.M. and 7–30 P.M. showing that active spawning took place in the early afternoon and the evening. The exact interval between oviposition and the above stage is difficult to guess, but taking the incubation period of other Clupeids as a criterion, it may be estimated that the stage referred to could not be roughly more than 4 to 6 hours after fertilization. The rate of development of the egg varied in different species of fish with the prevailing temperature. In the American Shad (*A. sapidissima*) the incubation period is reduced from 6 to 12 days in water of 52° to 65° F., to 3 days in waters at 75° F. (Leach, 1924). The eggs collected from the Daya River at about 3–30 P.M. on 16–9–1949 and at about 2–30 P.M. on 17–9–1949, had the blastodermal cap of cells, indicating an extended duration of breeding there also. The temperature of the water was not taken at the time of collection of the eggs; but being summer the temperature could inferentially be definitely higher than the Hooghly temperature in February, and hence the eggs would not have taken more than 2 to 3 hours to reach the above stage after fertilization, when compared to the rate of development of certain other brackish water fishes (Jones, 1937). The temperature of water in the Hooghly in Feburary ranged from about 23° to 25° C. During a cold spell, the temperature of the river water dropped to 19° C. and eggs were not present in the collections. It appears from the data available to us that *Hilsa* spawns towards the afternoon and evening.

Ovarian egg (Fig. 1 *a*).—Fully ripe oozing eggs of the VI stage are almost spherical with a diameter of about 0·70 mm. to 0·75 mm., of oily transparency, pale yellow in colour and demersal in character. In some Indian Clupeids (*Setipinna* and *Anchoviella*) the egg membrane is thin and does not swell up, while in *Hilsa* it is comparatively thick and swells up after fertilization.

Embryonic Development[5]

The only published account of the fertilization and early development of the egg of *Hilsa* is by Southwell and Prashad (1918) who have given the size of the ovarian, egg and the changes that take place immediately after fertilization.

The egg membrane of *Hilsa* which remains compactly round the vitellus before fertilization begins to swell up after fertilization as recorded by Southwell and Prashad (*op. cit.*) and as observed in other Clupeids like *Sardinella fimbriata*, *Alosa sapidissima*, *Sardina cærulea*, etc., and in certain carps. When

[5] See the addendum at the end of the paper.

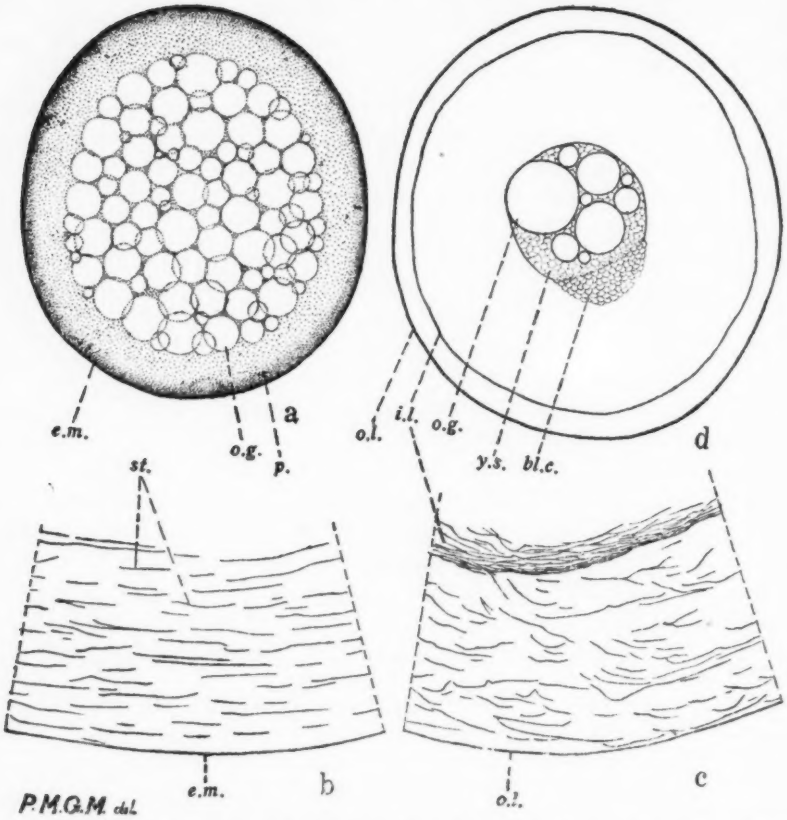

FIG. 1. *Hilsa ilisha* (Hamilton).—(*a*) Ripe ovarian egg (×80). (*b*) A portion of the fresh egg membrane of fertilized egg (× 80). (*c*) A portion of the egg membrane of the dead and preserved egg showing the double layer (×80). (*d*) Earliest stage of fertilized egg. Collected from the river showing the blastodermal cap of cells (Double layering is semi-diagrammatic). (× 30).

swollen, the egg of *Hilsa* is about 2 mm. in diameter with a pattern of fine streaks on its surface (Fig. 1 *b*). The egg membrane is elastic and, therefore, flattens out unlike that of the carp eggs, if they are placed on a slide. In eggs containing dead embryos, or in those under gradual fixation the membrane splits into 2 layers, one presumably representing an outer zona villosa, which remains in the expanded condition but with less distinct streaks, and the other, an inner zona radiata[6], with conspicuous and denser streaks (Fig. 1 *c*) due to contraction of the membrane; the space between

[6] In teleostean eggs the presence of only one egg membrane, *viz.*, the zona radiata, is generally recognised.

the 2 layers, being wider in some cases than shown in the figure. If the fixation is quick enough to prevent double-layering the whole egg membrane shrinks a little giving a puckered appearance. No change is seen in the egg membrane when hatching takes place. The egg, though demersal, is of a drifting type, being easily bouyed up. The swollen egg membrane provides a wide perivitelline space, which acts as a water-cushion against any shocks and as a buffer against any sudden variations in salinity and temperature.

The earliest stage (Fig. 1 *d*) of the embryo collected from both the Hooghly and Daya shows a blastodermal cap of cells and a wide perivitelline space. In healthy eggs no double membrane could be made out. The yolk is segmented as in a typical Clupeid egg and the numerous (maximum 20) oil-globules of varying sizes usually coalesce later to form a large and conspicuous globule. The apparently transparent embryo shows, on closer examination, first a faintly translucent blastodermal region and then the whole embryo. The embryo remains more or less at the centre of the large perivitelline space. The heavier blastodermal pole, and the lighter opposite pole with its oil-globules, remain at the lower and upper positions respectively following the natural laws of gravity and buoyancy. The embryonic development in general is typical of Clupeids. The main features observed are tabulated below:—

No. of hours after the earliest embryonic stage recorded	Developmental changes of the embryo at an average temperature of 23° C.
½ to 1	The blastodermal cells reduced to small size due to continuous division, giving rise to blastodermal cap, which by close apposition on to the deutoplast, spreads over it (Fig. 2 *a*).
4	Oil globules begin to coalesce together. Germ ring reaches equatorial region (Fig. 2 *b*).
5–6	Embryonic ridge seen as a long conspicuous thickening when three-fourths of the yolk is enveloped by the blastoderm (Fig. 2 *c*).
6–7	Blastopore closes. Myotomes become distinct. The number of oil-globules reduced to 2 or 3 large ones.
7–9	Optic vesicles perceptible. 10 myotomes distinct.
15	23 myotomes distinct, olfactory and auditory vesicles and caudal end marked off (Fig. 2 *d*).
16–19	Pulsations of the heart begin. Tail gets free from the yolk mass elongates and shows twitching.
20	Well marked features. Posteriorly elongated yolk. Long free tail. (Fig. 2 *e*).
23–26	Hatching takes place, the movements of the tail being helpful in rupturing the egg membrane.

It is usual to find one or more small oil-globules in the yolk a little removed from the large oil-globule which remain isolated throughout the embryonic and even during the early part of the prolarval development,

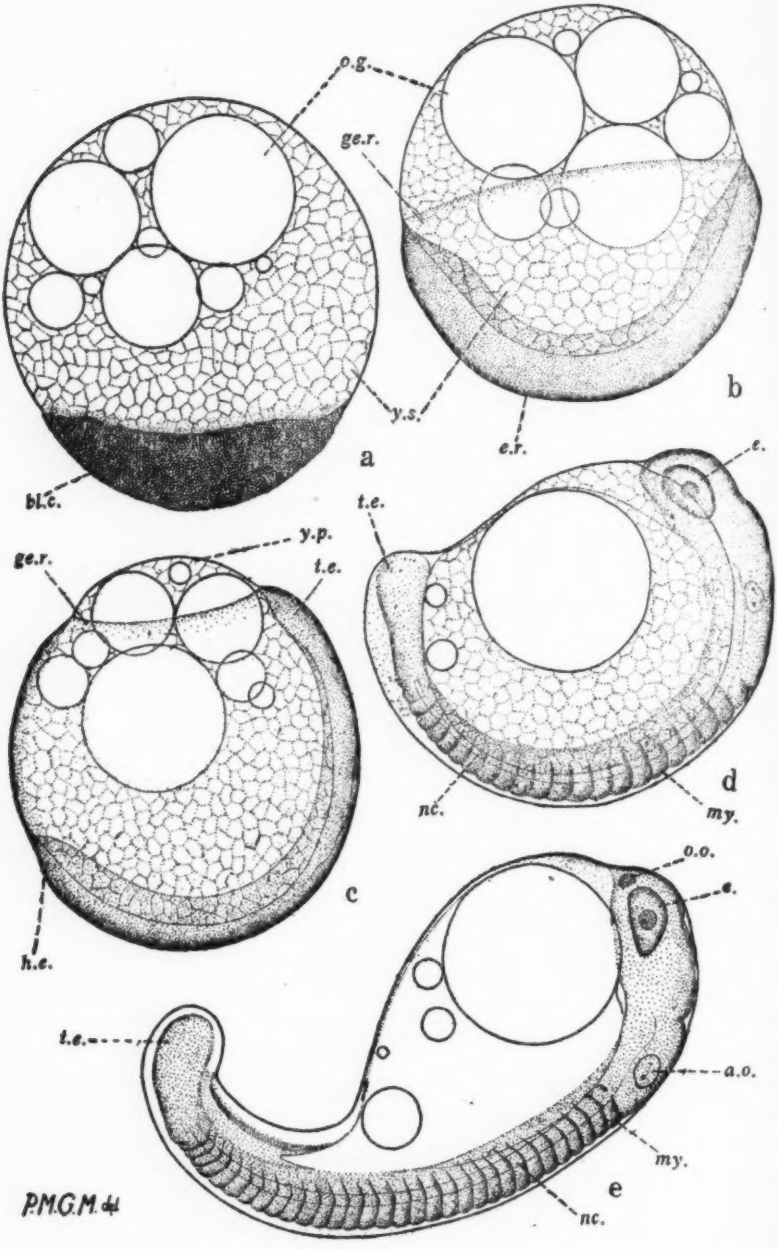
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FIG. 2

FIG. 2. *Hilsa ilisha* (Hamilton).—(*a*) Advanced stage of blastodermal cap just before the formation of the segmentation cavity (× 80). (*b*) Embryo showing the germ-ring at the equatorial position (×80). (*c*) Embryo an hour before the closing of blastopore (× 80). (*d*) Embryo 15 hours after the earliest stage observed (80). (*e*) Embryo about 20 hours after the earliest stage and 3-6 hours before hatching (×80) (Segmentation of the yolk not shown).

As already stated the rate of development varies with temperature; the lower the temperature, the longer the time taken for development. During February, the duration of development from the blastodermal to the hatching stage varied from 22 to 30 hours. Southwell's (1914) estimate of incubation period, *e.g.*, 9 days is obviously a conjecture based on the analogy of the American Shad, *Alosa sapidissima*. Raj (1941) found that in Madras the period varied from 12 to 18 hours. It is also interesting to note that 24 eggs collected on 11-2-1950 (*i.e.*, a day previous to the cold spell) failed to develop in the laboratory beyond the stage shown in Fig. 3 *d*. The temperature of the water in the glass jars in which the eggs were kept dropped from 24° C. to 16° C. by next morning. During this time the temperature of the river water dropped to 19° C.; but as this drop could only have been relatively gradual, the development of the eggs has probably been retarded. However, there appears to have been an interruption in breeding judging from the absence of eggs and yolked larvæ for a few days during the cold spell from 12th to 16th Feburary 1950.

Experiments were conducted in the laboratory with 2 lots of eggs (of stage Fig. 2 *a*). One lot of them was placed in a 1% solution of normal saline and the other in ordinary chlorinated tap water. In the former the eggs floated first at the surface and then sank gradually, while in the latter they behaved as in the river water. Both the lots of eggs hatched out on the next day and the larvæ lived for another day. Preliminary experiments with larvæ in tap water and filtered river water have shown that the larvæ could live for a day longer in the former than in the latter.

LARVAL DEVFLOPMENT[7]

First day.—The newly hatched larva (Fig. 3 *a*) which is 2·3 mm. long shows little movement as compared to other Clupeid larvæ such as *Setipinna phasa* (Jones and Menon, 1950 *a*). Like the egg it sinks to the bottom, keeping a more or less horizontal position except that the ventral part of

[7] Hubbs, C. L., 1944 [*Copea*, 1943 (4), p. 260] has recommended the division of the term 'larva' into the prolarva and the postlarva, the former denoting the yolked larva and the latter the larval stage after the absorption of the yolk, but applied only when the structure and form are still strikingly different from the later stages. [See also Jones, S., 'A note on the Terminology of the early developmental stages of fishes', *J. Zool. Soc. India*, II, (1), pp. 39–41, 1950.]

the head and the yolk are slightly tilted up. Occasionally the larva darts about for a while and comes to rest at the bottom.

The yolk mass extends to about the 10th myotome with a large oil-globule close to the head. One or more very small oil-globules may be present in the yolk. The eyes are unpigmented. The auditory organs are very distinct while the alimentary canal is not. The mouth and the anal openings are absent though the position of the latter is indicated near the 40th myotome. The pigment spots which are not clear at the time of hatching become gradually distinct in a few hours. Dorsally the larval fin-fold commences opposite the 2nd myotome and ventrally at the postero-ventral region of the yolk.

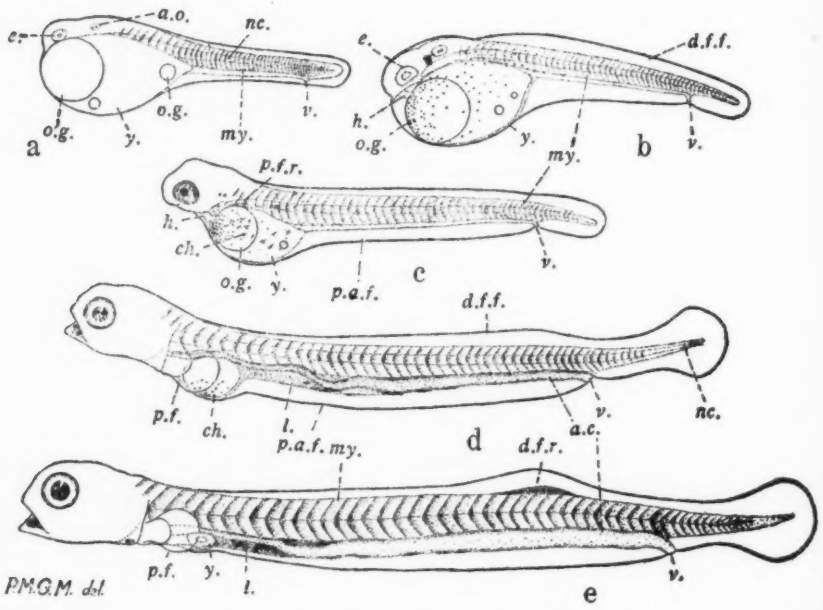

FIG. 3. *Hilsa ilisha* (Hamilton).—(*a*) Newly hatched larva (×18). (*b*) 12 hour old larva × 18). (*c*) 60 hour old larva (×18). (*d*) 5·6 mm. stage (×18). (*e*) 6·5 mm. stage (×18).

In about 12 hours (Fig. 3 *b*) the well-defined chromatophores can be seen to be distributed on the yolk, being denser towards the anterior and dorsal regions. There are a few on the trunk also, above the yolk. The postero-ventral region is almost devoid of pigment.

Second day.—The larva grows to 3 mm. in length showing some reduction in the amount of the yolk present and concentration of pigment spots in the region of the oil-globule. The eyes begin to turn dark. The caudal end is slightly expanded.

Third day.—The larva has grown to a length of 3·75 mm. The head, freed from the yolk mass, is slightly raised in front of the yolk, and the head region itself is considerably longer than in the earlier stages (Fig. 3 *c*). The eyes become darker. The rudiments of the pectoral fins arise from the sides of the body just above the oil-globule of the yolk. The chromatophores are concentrated together on the anterior region of the yolk due to its partial absorption. Those on the trunk region are much reduced in number. The alimentary canal appears as a fine tube without a distinct and visible anal opening. An indication of the formation of the mouth is seen along the ventral side of the head below the eyes. The hind margin of the operculum is faintly differentiated posteriorly, with the rudiments of the gills seen through the transparent operculum.

Fourth day.—The larva is slightly longer with the caudal end broader and rounded, and the mouth, the gills and the operculum better developed. The yolk is appreciably reduced and the eyes become darker still.

Fifth day.—The larva has attained a length of 5·6 mm. (Fig. 3 *d*) with most of the yolk fairly consumed, leaving only a large oil-globule. With the exception of a few elongated chromatophores on the ventral side of the alimentary canal all others become concentrated on the ventral region of the yolk. The distinctly formed mouth is now anterior in position. The almost straight alimentary canal, with a slight bend behind the liver is clearly differentiated. The rounded head end shows a tendency to become angular. The broad posteriorly rounded caudal end is devoid of any rays. The pectoral fins are better developed.

6–7 *mm. stage.*—By the time larva is 6·5 mm. (Fig. 3 *e*) long, the yolk is very much reduced with only a few black pigment spots along its ventral aspect. The larva begins to feed on minute copepods and other organisms in the water which could be seen under the microscope through the transparent walls of the alimentary canal. The first indication of a dorsal fin is seen between the 25th and the 31st myotomes, as a slightly raised basally thickened portion of the larval fin-fold, in which the radials begin to be perceptible. With the further growth of the larva the fin-rays are gradually formed. While these changes are taking place the fin becomes broader with a constriction behind it, and by the time the yolk is absorbed the posterior limit of the dorsal fin is completely cut off from the larval fin-

fold. The tip of the vertebral column which is almost straight till about the 7 mm. stage is tilted up with a slight thickening on its ventral region where hypurals appear and these become distinct by the time the fish has reached the postlarval (8 mm.) stage in which all the yolk is absorbed (Fig. 4 *a*). Rare instances of the yolk having been more or less completely absorbed in larvæ 7 mm. and 9 mm. long have been observed. Since the larva begins to feed even before the complete absorption of the yolk takes place the exact size at which it becomes a postlarva depends on the nutrition available during the prolarval stage.

The actual time taken for the complete absorption of the yolk could not be ascertained as the prolarva could not be reared to the postlarval stage. But from the observed rate of growth it may reasonably be presumed that the prolarvæ may take a week or a little over for the complete absorption of the yolk.

It is likely that the prolarvæ will survive the critical period under laboratory conditions if they are kept in clean circulating water and properly fed, taking care to see that they are not allowed to settle down at the bottom where they may be easily susceptible to bacterial and fungal attacks. Feeding and protecting the prolarvæ from any infection seem to be two important factors in their survival.

8 *mm. stage* (Fig. 4 *a*).—As the larva enters the postlarval stage when it is about 8 mm. long, the yolk is completely absorbed. It has a slender and compressed form with the caudal fin rounded and the head obtusely pointed. A few minute dots represent in some cases the last traces of 'yolk chromatophores'. One or more median circular black pigment spots could be seen just in front of the pectoral fins on the ventral side. 4 to 5 pigment spots are present behind the pectoral fins on each side of the liver. One or more elongated pigment spots are present in front of the anal opening and dorsal to the rectal portion of the alimentary canal at about the 39th and 40th myotomes. The dorsal contour of the head is distinctly convex. The mouth is terminal and the lower jaw slightly longer than the upper. A thin transparent maxilla is visible. The eyes are large, black and shining. The dorsal fin arises as an expansion of the narrow fin-fold on the dorsal side between the 25th and the 31st myotomes with indications of the formation of about 8 fin-rays. Just behind the posterior limit of the dorsal fin the larval fin-fold is completely cut off. The pre-anal fin-fold extends from the pectorals to the anus and is relatively broader than the dorsal fin-fold. The still rudimentary anal-fin has only a slight basal thickening. The posterior end of the vertebral column is slightly bent upwards, and on

the ventral region there is a slight thickening indicating the formation of the hypurals. The caudal rays are not yet distinct. The alimentary canal is nearly a straight tube except for a slight bend in the region of the air-bladder which may be seen between the 13th and the 18th myotomes. The liver is seen to extend up to the 11th myotome and just behind it, the alimentary canal shows the bend. The anus is situated near the 40th myotome, far behind the posterior limit of the dorsal fin. The larva feeds on diatoms and copepods and sometimes whole copopods may be seen inside the alimentary canal.

10 *mm. stage* (Fig. 4 *b*).—In general shape this stage is more or less similar to the previous one. The anterior end of the larval fin-fold on the dorsal side shows a tendency to atrophy, whereas, in the pre-anal fin-fold there is no appreciable change. The better developed dorsal fin with about 12 to 13 incompletely developed rays extends between the 23rd and the 29th myotomes; the last ray shows the double nature as in the adult fin. In the anal fin there are faint indications of the formation of the radials. A tendency towards the heterocercal condition, is noticed at the posterior end of the caudal fin. The rudiments of 19 caudal fin-rays could be clearly made out. The anal opening is between the 38th and the 39th myotomes. Two or three pigment spots are seen on the ventral margin, within the gill cover. The external pigment spots in the region of the liver are clearer than in the earlier stage. There is an irregular row of minute pigment spots along the ventral line below the alimentary canal. Apart from these, a few pigment spots are seen internally in the region of the air-bladder. A few are present on the caudal fin and a large stellate one immediately behind the anal fin.

12 *mm. stage*.—The convexity of the anterior end of the head is less than in the previous stage. The gill rakers can be seen through the operculum. The dorsal fin-fold has atrophied to about the 10th or the 11th myotome and the dorsal fin with about 16 rays starts from about the 22/23rd myotome and ends in the 28th. The posterior end of the anal fin shows a notch separating it from the fin-fold behind, and the formation of the fin-rays is visible. The hypurals and the 19 caudal fin-rays are distinctly seen. The fin-rays shows articulations and are grouped into 2 sets of 10 and 9 attached to the dorsal and ventral sets of hypurals respectively. There are within a few chromatophores the body, but visible through the body wall along the dorsal margin of the alimentary canal and a few more above the notochord between the caudal and the anal fins.

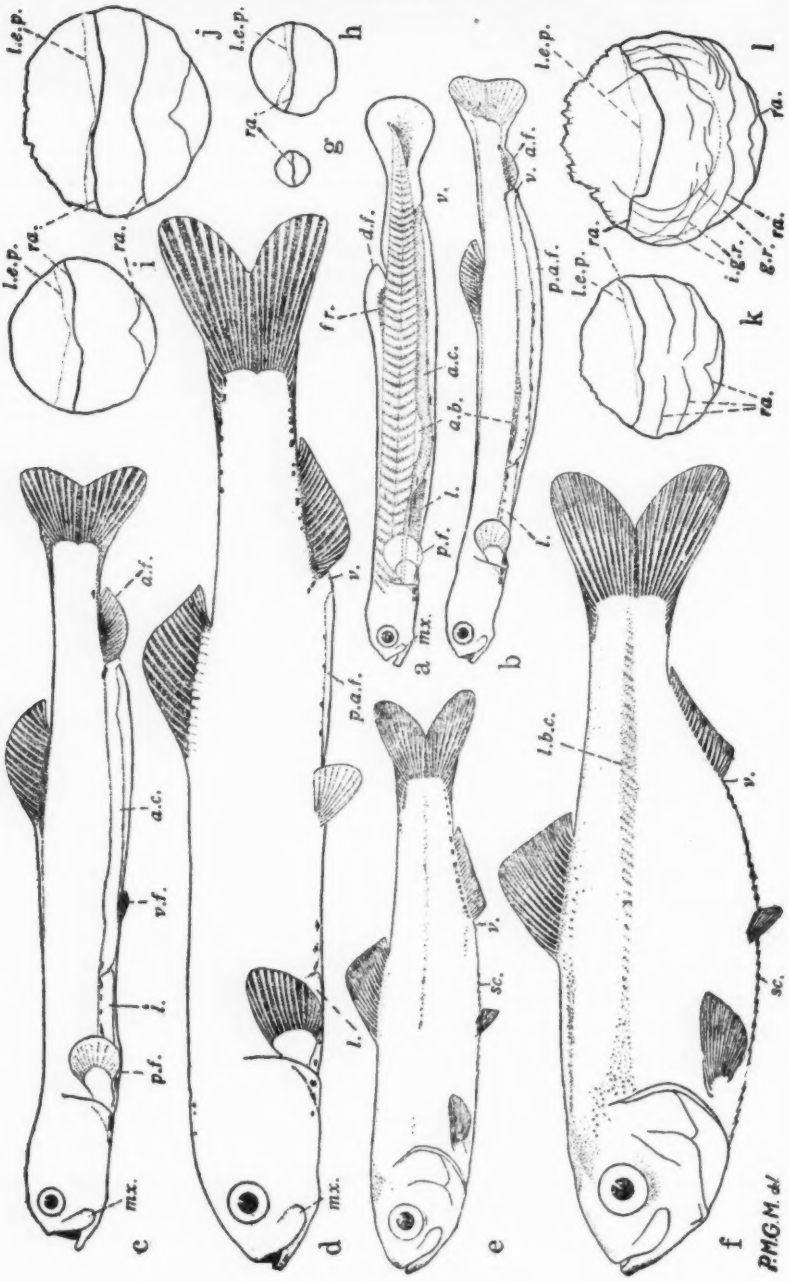
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FIG. 4

*FIG. 4. *Hilsa ilisha* (Hamilton).—(*a*) 8 mm. stage (×10). (*b*) 10 mm. stage (×8). (*c*) 14 mm. stage (×8). (*d*) 18 mm. stage (×8). (*e*) 20 mm. stage (×4). (*f*) 30 mm. stage (×4). (*g*) Scale of 21 mm. stage (× 9½). (*h*) Scale of 50 mm. stage (× 9½). (*i*) Scale of 75 mm. stage (×9½). (*j*) Scale of 101 mm. stage (×9½). (*k*) Scale of 162 mm. stage (× 5). (*l*) Scale of 213 mm. stage (×5).

* (The internal chromatophores, and the articulations and branchings of the fin-rays are not shown in the figures.)

14 *mm. stage* (Fig. 4 *c*).—The anterior end of the head is more pointed than in the earlier stages. In the alizarin preparations the formation of the vertebral spines are seen in the posterior half of the body. An important additional feature in the development is the indication of the ventral fins, which commence as thickenings just after the 12 mm. stage. These appear like buds at the 13 mm. stage and, by the time the larva grows to 14 mm. in length, distinctly project out from the body between the 12th and the 13th myotomes. The anterior portion of the dorsal fin-fold has atrophied and the dorsal fin with 18 rays starts near about the 19th myotome and extends up to the 28th myotome. The pre-anal fin-fold has disappeared in front of the ventral fin, and posteriorly it is slightly narrower than in the earlier stages. By this time the development of the anal fin has also advanced considerably and 18 fin-rays could be made out dinstictly. The caudal fin, by this time shows the typical homocercal condition of the adult and has dorsally 4 epaxial fin-rays, and ventrally below the 19 principal rays, 3 short rays. The anus has shifted considerably forwards and is situated between the 34th and the 35th myotomes and decidedly behind the posterior limit of the dorsal fin. Two additional rows of internal chromatophores, one along the dorsal margin of the alimentary canal extending backwards up to the anus and another above the vertebral column behind the dorsal fin make their appearance. With the larva becoming relatively stout the internal organs are not so easily made out as in the earlier stages.

16 *mm. stage*.—The body of the larva is uniformly wide up to the anal region. The anterior end of the body is more angular than in the previous stages. The jaws are well developed with the lower jaw bones having fine conical projections on their anterior edge. The dorsal fin-rays now extend between the 18th and the 28th myotomes, and the full complement of 19 rays of the adult fish is developed; all except the anterior 3–4 rays show the articulations and branchings. In the anal fin 19–20 fin-rays are present. The ventrals have all the 9 rays developed in each and in the pectorals the formation of 8–10 rays can be made out. In the caudal fin the development of some more unbranched and unarticulated epaxial and hypaxial rays is noticed. Just behind the region of the liver the alimentary canal shows a distinct fold, and the anus has shifted forwards to about the 32nd myotome.

The larval fin-fold has disappeared in all regions except between the ventrals and the anus. The row of pigment spots inside the body seen behind the dorsal fin in the previous stage has continued forwards almost to the anterior half of the region below the dorsal fin. There are a few small stellate pigment spots on the caudal fin and the caudal peduncle, and some at the base of the anal fin. Towards the postero-dorsal region of the head also a few minute stellate chromatophores have made their appearance. The internal row of spots along the dorsal margin of the alimentary canal now extends from behind the operculum to the anus, and inside the operculum also a few stellate spots are visible.

18 *mm. stage* (Fig. 4 *d*).—The intestine is coiled on itself and is not so clear as in the earlier stages though undigested copepods are seen inside. The larval fin-fold is narrower than in the previous stage. The anus is situated near the 30th myotome. The radials of the dorsal fin are better developed than those of the anal. A few more additions of the epaxial and the hypaxial rays could be noticed in the caudal fin. The abdomen is not yet keeled and the scutes have not been formed. In the pectorals 10 rays are distinctly seen. The pelvic girdle is in the course of formation. In the 18 mm. larva there is a definine increase in the number of pigment spots and the internal row above the vertebral column has slightly extended forwards. At the base of the epaxial rays a few stellate chromatophores are visible. There is a minute black spot at the base of each dorsal fin-ray. A few chromatophores have made their appearance on the lower jaw also. With further increase in the length of the larva, it is no more an 'early postlarva,' and this stage marks an important transitional phase in the course of the development of the fish.

By the time the larva attains a length of about 20 mm. (Fig. 4 *e*) it has assumed a shape characteristic of the Clupeoids and has definitely passed the early postlarval stage to the late postlarval phase leading gradually on to the 'juvenile' fish. The posterior margin of the operculum is more or less curved as in the adult. The origin of the dorsal and ventral fins are almost in a line. The dorsal fin-rays arise above the 12th or the 13th segment. The anus is near the 29th segment. The full complement of rays of all the fins including the pectoral and the anal is present. The vertebræ are all well demarcated, with the plural ribs arising from them. The ventral region of the abdomen is somewhat keeled and there are 5 to 7 scutes in front of the ventral fin. A row of minute chromatophores may be seen along the dorsal edge of the larva and more and more stellate chromatophores are seen at the base of the epaxial fin-rays. Concentrations of pigment are formed in the postero-dorsal region of the head. Scales begin to develop

and are distinctly visible when the postlarva is about 21 mm. long. With further increase in length more and more pigment spots are developed inside the body, and a row on the dorsal and another on the ventral aspect of the vertebral column are distinctly seen by the time the larva is about 24 mm. in length. Numerous minute chromatophores are distributed over the dorsal aspect of the head rendering it dark. This becomes more and more marked with further development, being continued behind as a regular line or row of pigment spots extending to the caudal fin along the dorsal margin of the larva. Along the base of the anal fin reaching up to the caudal there is another row of pigment spots. The dorsal fin moves further forward, with its anterior limit in advance of that of the ventral. About 27 scutes have already made their appearance and the vertebral column and ribs show progress in development. When the larva attains about 26 mm. in length its shape is roughly of the adult and more pigment spots have developed on the body giving rise to the lateral band seen in the juvenile specimens. There are 30 scutes on the ventral edge of the abdomen. The full complement of the adult fin-rays is developed in all the fins, and the dorsal commences from about the 9th segment. With increase in length to about 30 mm. (Fig. 4*f*) all the 31 scutes that are found in the adult fish are fully formed and the fish has completed the postlarval stage and is now almost a 'juvenile' having most of the external distinguishing characters of the adult. The opercular margin is curved as in the adult. The dorsal fin is at about the 8th segment and the anus at about the 30th. In the juvenile fish, unlike the adult, the markings on the sides of the body are more prominent and there is a distinct lateral band which is characteristic of the young.

The food during the larval stages consists mainly of diatoms and copepods. With further growth, the juvenile fish takes on to a variety of other planktonic organisms.

The essential changes in the course of further development of the fish have been given by Nair (1939). The anus gradually shifts backwards till it comes to the 34th segment in the adult. The band on the side becomes darker and broader, and after it is 41 mm. in length concentrations of pigment appear in the form of dark blotches along the band which are the characteristic markings of *Hilsa*, more distinct in the young rather than in adults. Such young forms up to about 6 inches are called "*Hilsa-koira*" in W. Bengal and they are the counterpart of the '*Jatka*' fish of East Bengal described by Hora and Nair (1940 *b*).

The average rate of growth during the first year of life of the fish is about 25 mm. a month as recorded by the Madras Fisheries Department.[8]

[8] *Madras Fisheries Department Administration Report for* 1936–37.

According to Hora (1938) the brood of July-August may attain a length of 140–150 mm. by the 3rd week of November. Hora and Nair (1940 *a*) as a result of their observations made in the settling tanks and filter beds of the Pulta Water Works record a growth of 35 mm. a month during November while in the summer months the rate is as high as 50 mm. Our observations made at the Chilka Lake have shown that the early lot, *i.e.*, July-August brood is about 110 to 133 mm. by the 1st week of December. The above rate of growth may not be maintained throughout the life of the fish, as it is only natural that there should be an arrest in growth at the time of spawning.

Though as stated already some data about the rate of growth per month during the first year of life are available, scale studies have not been of much help in the determination of the exact age of the fish. Chacko and others (1948) report that the number of radii represents the length of the fish in inches and that the age of the fish based on growth records of an inch per month can also be roughly determined by counting the number of radii. Even if this holds good for the first few radii it is obvious that the fish cannot grow throughout its life in the manner presumed by them. According to the above theory, if the fish is to live for 5 to 7 years as estimated by Hora and Nair (1940 *a*), the scale should show dozens of radii not to speak of the enormous length, the fish should attain! Scales begin to appear when the fish is about 21 mm. in length and only the primary radius is present up to 50 mm. stage (Figs. 4 *g* and *h*). One additional radius is seen in the scale of a fish of about 75 mm. (Fig. 4 *i*), 2 more in the scale of 101 mm. fish (Fig. 4 *j*) and 3 more in the scale of 162 mm. fish (Fig. 4 *k*), and 4 more in a scale of 213 mm. fish (Fig. 4 *l*). The last fish is an oozing male caught from the Hooghly about 2 miles above Pulta on 3–2–1950, and the scale as shown in the figure has one distinct "growth-ring" and two incomplete rings. We have found that, though the number of radii increases corresponding to the increase in size of the scale and length of the fish, it is difficult to draw a correct relationship between the radii and the age of the fish and at the same time it has not been possible to interpret the exact significance of "growth-rings" on the scales which, as Prashad, Hora and Nair (1940) say 'is still obscure'.

The total span of life of the fish is not known, but Hora and Nair (1940 *a*) as a result of scale studies give a period of 5 to 7 years and try to explain the 5-year cycle of *Hilsa* in Bengal (*i.e.*, the abundance of the fish once in 5 years) due to the possibility of the bulk of the stock of fish belonging to one bumper year becoming sexually mature in the 5th year of its life

as in the case of the European Herring. The above is only a tentative inference, as, in the tropical waters, for reasons well known, a correct interpretation of the markings on the scale is difficult. Our present data do not permit us to draw any definite conclusions.

Day (1873 *a*) surmised that the first maturity of the fish may be earliest at the end of the first year or at the beginning of the second year. Our general observations on over a 1,500 specimens of the fish in the Hooghly, Chilka Lake and the Mahanadi corroborate this, especially in the case of males, 8½ to 10 inches long, as the fish of this size could hardly be over an year old. The first maturity of the females may be during the second year, since the smallest female specimens with mature gonads were a little longer than the males, *i.e.*, 10½ to 12 inches.

Migratory Movements

Though considerable information has been gathered on the ascent of *Hilsa* in our rivers, our knowledge of the life and wanderings of the fish, in the estuaries and coastal waters is very meagre. In spite of intensive studies on the American Shad, little information on this aspect is yet available (*vide* Johnson, 1938). As first indicated by Day (1873 *b*) in the case of *Hilsa*, all workers agree that the fish spends part of its life in the sea, not far from the shallow coastal belt. The exact age when the fish goes into the sea is not definitely known. According to Raj (1937) "the fish spend the first years of their life in the lower reaches of rivers and go to the sea in third years". The observations of Prashad, Hora and Nair (1940) show that the fish migrates into the sea during the first year of its life and that it does not move far out into the sea, but moves along the foreshore waters. Mazumdar (1939) refers to the presence of *Hilsa* 9 to 10 inches long along the Chittagong Coast, and our find of young *Hilsa* about 2 inches from near Jaunput on the Contai Coast early in June 1949 gives support to the finding of Prashad, Hora and Nair (*op. cit.*) in respect of its seaward movement. It may be that the effluence of several large rivers reduces the salinity of water at the head of the Bay of Bengal much below that of the open sea bringing about estuarine conditions. The record of *Hilsa* by Raj (1932) in large numbers in the Palk Bay from November to May is of interest, as the salinity in this area should be typical of the sea. Further studies in this region are likely to yield valuable information regarding the movement of the fish towards the sea.

It is likely that after the floods subside, the *Hilsa* in the estuaries react to the currents prevalent in the coastal waters and move about there.

The factors influencing the above migration form subjects of interesting study.

General Remarks

Hilsa is one of those fishes, the conservation of which is of primary importance; and to achieve this a fuller knowledge of their spawning habits and life-history is very essential. The object of the present paper in delineating the gross features in the embryonic and larval development of this fish in fair detail is that this may prove useful to other workers in recognising similar stages in the field and in locating the spawning grounds of the fish. Generally in the case of migratory fishes the presence of gravid or young fishes in a locality does not necessarily mean that it is also its spawning ground. The criterion for determining the spawning grounds should at least be the procurement of the laid eggs. The flow of water in rivers and tidal areas renders the location of the actual spawning grounds rather difficult since eggs are drifted to distant places.

A fact that has been brought to light by the observations of the Fisheries Department of Madras is that even in rivers, the lower reaches of which are spanned by anicuts and weirs, a regular fishery exists, thereby showing that the fish is breeding even below the dams and anicuts. It has been found in the Mahanadi also that in spite of the anicuts at Cuttack and Naraj a regular fishery exists in the lower reaches of the river.

It is beyond the scope of this paper to go into the conservation aspect of the *Hilsa* fishery, but it may not be out of place to refer to some of the crucial points on the subject. We have 3 recent recommendations regarding the conservation of the *Hilsa* fishery namely, (1) Hora's (1938) suggestion that the protection of the brood fish and young should be aimed at rather than the construction of hatcheries and fish passes, (2) Raj's (1941) emphasis on successful artificial propagation of *Hilsa* to supplement the stocks in the rivers, and (3) Devanesan's (1942) suggestion that the brood should be protected below the anicuts for a specified minimum distance by the enforcement of rules regarding a closed period for fishing.

The fact that *Hilsa* breeds in sufficiently large numbers in the lower reaches of the rivers is an indication that the first recommendation, namely, to protect the brood fish and the young should be preferred to the other costlier measures. A fair-sized *Hilsa* 16 to 18 inches long contains about 1 million eggs, and consequently any measure that would facilitate the spawning of 100 fish is preferable to a hatchery liberating 100 million fry—even if such a hatchery was a practical proposition—the fate of which is uncertain. In this connection we may point out that the actual damage

done to the potential fishery by the operation of the small-meshed nets is far more alarming than has been realised (Hora, 1938). In the 'Bhin-jals' operated by the Hooghly fishermen we have seen, especially during the months of October and November, large numbers of *Hilsa* larvæ jammed together into an almost pulpy mass along with other fishes and their larvæ. As the cod end of the net is blocked by the entry of large-sized fish as soon as it is operated the exit of smaller fish that follow thereafter, through the meshes into the river, is rendered difficult. We have collected large quantities of larvæ almost 50,000 to a seer, from such nets. The damage caused to the fry and fingerling sizes is also enormous.

A proper survey of the actual spawning grounds of the *Hilsa* in the lower reaches of all the rivers in the country, in which it is found is urgently required. Devanesan's (1942) doubt that the young *Hilsa* would not be able to survive the action of saline water of the estuaries does not seem to be well-founded, as our observations show that they can tolerate appreciable variations in salinity. Large numbers of postlarvæ which drift down the current into the estuaries probably continue to develop there; but the fate of eggs and the prolarvæ is difficult to surmise.[9] It has not yet been ascertained whether the fish breed in the lower reaches of the estuaries also where the water is always saline, and whether the young fish recorded from there are actually from the larvæ that hatch out within the freshwater limits.[10]

There are admittedly considerable gaps in our knowledge of the life-history of *Hilsa* which require the cordinated efforts of a team of research workers to fill (*vide* Hora, 1940, p. 108 and Job, 1942, p. 429). Thus the magnitude of the *Hilsa* problem is great and as a result of the present investigations, only one aspect of the problem has been partially solved, and there remains a great deal more to be done both from the purlely scientific as well as the economic points of view.

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[9] In one of the routine plankton collections made at Diamond Harbour, on 30–3–1950, by Mr. J. C. Malhotra of this Station, a yolked larva about 2 days old was obtained. This is of significance since the salinity of the water at the time of collection was 10,000 p.p.m.

[10] It is said that the Twait Shad spawns in the Elbe near the mouth of the river, just above the brackish water, while the Allis Shad ascends the River Rhine to spawn as far as Basle, and in the Elbe above Dresdon. (Cunningham, J. T., *The Natural History of the Marketable Marine Fishes of the British Islands*, London, 1896, p. 179.)

The possibility of the presence of two or more races of *Hilsa*—physiological races—spawning in the different reaches in the same river as pointed out by Jenkins, J. T. [*Curr. Sci.*, 1940, **9**, (5): 243] also remains to be studied.

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Our thanks are due to Dr. T. J. Job, Chief Research Officer, Central Inland Fisheries Research Station, for the keen interest he has taken in the work and for his valuable criticism.

SUMMARY

The available information on the life-history of *Hilsa ilisha* (Ham.) is given briefly and discussed. The collections of eggs and early larvæ from the Hooghly River in West Bengal and from the Daya River (Orissa) are recorded. The spawning habits are given. The unfertilized egg which is 0·70 to 0·75 mm. in diameter, is almost spherical, transparent and demersal though it contains many oil-globules. A fair-sized female *Hilsa* is estimated to contain 1 million eggs. Spawning seems to take place generally towards the evening. Stages of embryonic development up to the time of hatching are figured and described. At a water temperature of about 23° C. to 25° C., the egg hatches in about a day. The eggs hatch out just as well in 1% normal salt solution and in ordinary chlorinated tap water as in the river water. The newly hatched larva is 2·3 mm. long. The larvæ lived under laboratory conditions for a maximum period of 5 days attaining a length of 5·6 mm. Yolk is completely absorbed when it is about 8 mm. long. The larval development of the fish till it becomes a juvenile is described and figured. From available records it appears that growth during the first year is roughly at the rate of an inch per month. Our observations show that the fish descend into the sea during the first year of their life. The need for further study on the bionomics of the fish is indicated. The great damage done to the *Hilsa* fishery by the capture of the countless fry and fringerlings of the fish in the tidal areas is stressed and the urgent need for the protection of the fry and the early stages is emphasized. The desirability for a proper survey of the spawning grounds in the rivers and further investigations into the fate of the egg and the larvæ that drift down the estuary is indicated.

REFERENCES TO LITERATURE

Chacko, P. I., Zobairi, A. R. K. and Krishnamurthi, B. "The Radii of Scales of *Hilsa ilisha* (Hamilton) as an index of Growth and Age," *Curr. Sci.*, 1948, **17**, 158–59.

Chacko P. I. and Ganapati, S. V. .. .. "On the Bionomics of *Hilsa ilisha* (Hamilton) in the Godavari River," *Journ. Madras Univ.*, 1949, **18**, 16–22.

Day, F. .. .. .. *Report on the Freshwater Fish and Fisheries of India and Burma*, 1873 *a*, pp. 22, 23, 35 and 36.

—— .. .. .. *Report on the Sea Fish and Fisheries of India and Burma*, 1873 *b*, pp. 24, 25 and App. pp. i, xxxi, cxxi, cxxii.

—— .. .. .. *Fishes of India*, 1878, pp. 638, 640.

Devanesan, D. W. .. .. "Research Work on the *Hilsa*," *Curr. Sci.*, 1942, **8**, 126.

—— .. .. .. "Weirs in South India and their effect on the Bionomics of the *Hilsa* in the South Indian Rivers—The Godavari, the Kistna and the Cauvery," *Ibid.*, 1942, **11**, (10), 398.

Hamilton, F. .. .. *An Account of the Fishes found in the River Ganges and its branches*, Edinburgh, 1822, 243–46.

Hora, S. L. .. .. .. "A preliminary Note on the Spawning grounds and Bionomics of the so-called Indian Shad *Hilsa ilisha* (Hamilton) in the River Ganges," *Rec. Indian Mus.*, 1938, **40**, 147–58.

—— .. .. .. "Life History and Wanderings of *Hilsa* in Bengal Waters,' *Journ. Roy. Asiat. Soc. Bengal*, Science, 1940, **6** (2), 93–112 (1941).

—— and Nair, K. K. .. "Further Observations on the Bionomics and Fishery of the Indian Shad, *Hilsa ilisha* (Hamilton), in Bengal Waters," *Rec. Indian Mus.*, 1940 *a*, **42**, 35–50.

———— .. .. "The Jatka fish of Eastern Bengal and its significance in the Fishery of the so-called Indian Shad, *Hilsa ilisha* (Hamilton)," *Ibid.*, 1940 *b*, **42**, (4) 553–65.

Jenkins, J. T. .. .. "Spawning of *Hilsa*," *Curr. Sci.*, 1938, **7**, 251–252.

Job, T. J. .. .. .. "*Hilsa* Investigations in Bengal Waters," *Sci. and Cult.*, 1942, **7**, 427–29.

Johnson, Fred R. .. .. "Marketing of Shad on the Atlantic Coast," *Investigational Rept.*, 1938, No. 38, **2**, *U.S. Dept. Comm.*, Washington.

Jones, S. .. .. .. "Observations on the Breeding Habits and Development of certain Brackishwater Fishes of Adyar, Madras," *Proc. Ind. Acad. Sci.*, 1937, **5** (6), 261–89.

—— and Menon, P. M. G. .. "Spawning Habits and Development of the Gangetic Anchovy, *Setipinna phasa* (Hamilton)," *Curr. Sci.*, 1950 *a*, **19**, 25.

———— .. .. "Spawning of *Hilsa ilisha* (Hamilton) in the Hooghly River," *Sci. and Cult.*, 1950 *b*, **15** (11), 443–44.

Leach, G. C. .. .. "Artificial Propagation of Shad," *U.S. Bur. Fish. Doc.*, 1925, No. 981, 459–86.

Mazumdar, C. H. .. .. "Foreshore Fishing in the Eastern Part of the Bay of Bengal," *Sci. and Cult.*, 1939, **5**, 219.

Nair, K. K. .. .. .. "On Some early stages in the Development of the so-called Indian Shad, *Hilsa ilisha* (Hamilton)," *Rec. Indian. Mus.*, 1939, **41**, 409–18.

Prashad, B. .. .. .. *Annual Report of the Dept. Fish. Bengal, Bihar and Orissa for the year ending* 31*st March*, 1919, p. 4.

Prashad, B., Hora, S. L. and Nair, K. K. .. "Observations on the Seaward migration of the so-called Indian Shad, *Hilsa ilisha* (Hamilton)," *Rec. Ind. Mus.*, 1940, **42**, 529–52.

Raj, B. Sundara .. .. "On the habits of the *Hilsa* (*Clupea ilisha*) and their artificial propagation in the Coleroon," *Journ. Proc. Asiat. Soc., Bengal* (N. S.), 1917, **13**, p. clxxxiv.

*—— .. .. .. *Madras Fisheries Department Administration Report for the year* 1930–31, 1932, p. 32.

*—— .. .. .. *Ibid.*, 1935–36, 1937, p. 38.

Raj, B. Sundara .. .. "Dams and Fisheries; Mettur and Its Lessons for India," *Proc. Ind. Acad. Sci.*, 1941, **14** B, (4), 341–58.

Southwell, T. .. .. "Report on *Hilsa*-hatching operations conducted at Monghyr, Bihar, during August, September and October, 1912," *Bull. Dept. Fish. Bengal, Bihar and Orissa*, 1914, No. 4, 1–5.

——— and Prashad, B. .. "On *Hilsa* Investigations in Bengal, Bihar and Orissa," *Ibid.*, 1918, No. 11, 6–12.

*Wilson, H. C. .. .. "Artificial propagation of *Hilsa* in the Coleroon," *Govt. of Madras, Revenue Dept.*, G.O., 1219, 1909 (5th May).

* Not directly consulted by the authors.

Key to Lettering

a.b., Air bladder; *a.c.*, Alimentary canal; *a.f.*, Anal fin; *a.o.*, Auditory organ; *bl.c.*, Blasto; dermal cap of cells; *ch.*, Chromatophore; *c.f.*, Caudal fin; *d.f.*, Dorsal fin; *d.f.f.*, Dorsal fin-fold-*d.f.r.*, Dorsal fin rudiment; *e.*, Eye; *e.m.*, Egg membrane; *e.r.*, Embryonic ridge; *f.r.*, Fin radial; *g.r.*, Germ ring; *g.r.*, Growth ring; *h.*, Heart; *h.e.*, Head end of embryo; *i.g.r.*, Incomplete growth ring; *i.l.*, Inner layer of egg membrane; *l.*, Liver; *l.b.c.*, Lateral band of chromatophores; *l.e.p.*, Limit of exposed portion of scale; *my.*, Myotome; *mx.*, Maxilla; *nc.*, Notochord; *o.g.*, Oil-globule; *o.l.*, Outer layer of egg membrane; *o.o.*, Olfactory organ; *p.*, Protoplasm; *p.a.f.*, Pre-anal fin-fold; *p.f.*, Pectoral fin; *p.f.r.*, Pectoral fin rudiment; *ra.*, Radius; *sc.*, Scute; *st.*, Streaks on the surface of egg membrane; *t.e.*, Tail end of embryo; *v.f.*, Ventral fin; *v.*, Vent; *y.*, Yolk; *y.p.*, Yolk plug; *y.s.*, Yolk segment.

ADDENDUM

An article by Kulkarni,[11] which has come out since the communication of this paper for pubication, requires special mention here. It contains an interesting account of the *Hilsa* fishery in the Narbada River (Bombay coast), the collection of fertilized eggs of *Hilsa* from the tidal portion of the river and the artificial fertilization and early development of the fish till the 4th day after hatching. Except for certain very minor differences between our observations, which are only usual in a work of this type, the two accounts are both complementary and supplementary, and along with the information contained in the Administration Reports of the Madras Fisheries Department and contributions of Hora (1938) and Nair (1939) and Prashad, Hora and Nair (1940) will go a great deal in giving a fair idea of the life-history of the fish.

The diameter of 1·3 to 1·5 mm. given by Kulkarni (*op. cit.*) for the freshly laid eggs is much higher than that given by Southwell and Prashad (1918), Chacko and Ganapati (1949) and by us in this paper and is probably inclusive of the preliminary swelling that quickly follows extrusion as evident from his figures. The yolk of the *Hilsa* egg is actually segmented as that of

[11] Kulkarni, C. V. (1950) "Breeding Habits, Eggs and Early Life-History of the Indian had, *Hilsa ilisha* (Ham.) in the Narbada River," *Proc. Nat. Inst. Sci., India*, **15** (3), 169–76.

the eggs of the American and European shads and other Clupeoid fishes. We differ from his view that the large conglomeration of oil globules in the centre of the eggs 'gives the appearance of segmented yolk which is considered by Delsman (1926) as characteristic of all Clupeoid eggs' (Kulkarni, *op. cit.*, p. 174). We have had the opportunity of examining a large number of eggs of both estuarine and marine Clupeoid fishes and have found that the yolk is invariably segmented in all as has been recorded by various other workers. As regards the egg membrane, we could distinguish clearly the double layering only in preserved eggs. The rate of development is quicker in the Narbada at a temperature of 27·5° to 28·5° C. in the month of August and the incubation period is 18 to 26 hours. In Madras where the temperature may be slightly higher hatching takes place in about 12 to 18 hours (Raj, 1941).

Chacko and Krishnamurthy[12] in a recent note say that rings in the scales of *Hilsa* denote periods of spawning and that 'from the number of rings present on a scale the number of times the fish had spawned may be determined'. In the absence of any specific interpretation of the markings in the scales the possibility that the rings may denote periods of spawning cannot be entirely ruled out; but it would be misleading to take this view for granted without adequate evidence. It may also be mentioned here that the 'biometrical' data presented by the above authors appear to militate against their own previous 'radii' hypothesis (Chacko, *et al.*, 1948) referred to on page 118 of this paper (15–2–1951).

[12] Chacko, P. I., and Krishnamurthy, B. (1950), "A biometrical study of *Hilsa ilisha* (Ham.) in the Godavari River," *J. Bombay Nat. Hist. Soc.*, **49** (2), 315 & 316.

SEASONAL VARIATION IN THE CHEMICAL COMPOSITION OF MACKEREL (*RASTRELLIGER KANAGURTA* RUSSEL)*

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Introduction

The variation in chemical constituents such as water, protein, fat and occasionally ash of any fish is attributed to changes in growth, size, maturity, sex, food of the fish and locality from which it is caught.

The greatest variable among the above mentioned constituents is fat or oil contents (Atwater, 1888; Milroy, 1908; Johnstone, 1915; Bruce, 1924; Elsaby, 1937; Wilson, 1939; Stansby and Lemon, 1941 and Riegol, 1948). Hornell and Ramaswami Naidu (1924) working on the Indian Oil Sardine stated that the variation in fat was attributable to the presence or absence in the plankton of two important varieties—the dinoflagellates and copepods. Devanesan (1943) also confirmed the above view. In this present report of ours, we have not taken up this aspect and have confined ourselves purely to the variation in the chemical constituents.

The systematic study of seasonal variation in the chemical composition of fish flesh with particular reference to the variation in the oil content is an interesting one. The Malabar shoaling fish—the Mackerel (*Rastrelliger kanagurta* Russel)—is the subject of our study. Except for Hornell and Ramaswami Naidu's (1924) paper, no systematic study appears to have been published up to now. Even this work which records only a field observation on the fat variation of the Oil Sardine, cannot be said to constitute a regular chemical analysis. The present study has been in progress since June 1946 and it is intended in this paper to embody a preliminary report of the progress of work done so far.

Material and Methods

The mackerels in a fresh condition were obtained from boats which fished within a five mile radius off the coast of Kozhikode and about 10 to

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TABLE I. *Chemical Composition of Mackerels* (1946–49)

Date	Average length in cm.	Average weight in gm.	Water %	Protein %	Fat %	Ash %
8–7–46	21·0	105	77·43	18·92	0·69	1·83
1–8–46	23·5	91	71·85	21·78	1·61	1·46
20–8–46	19·5	88	77·05	20·35	1·78	1·58
9–9–46	13·5	37	76·51	19·63	1·45	1·40
10–9–46	14·5	38	75·67	20·80	2·83	1·62
1–10–46	17·5	67	74·82	19·43	1·86	1·53
14–10–46	18·0	67	77·02	19·71	1·33	1·48
1–11–46	18·0	67	77·46	19·87	0·81	1·58
14–11–46	17·5	61	78·20	19·23	1·09	1·65
2–12–46	17·5	60	77·18	19·54	1·00	1·52
17–12–46	17·8	60	77·94	19·43	0·71	1·70
8–1–47	18·5	63	75·08	20·81	0·80	1·38
20–1–47	18·5	61	76·52	20·60	0·63	1·53
17–2–47	19·0	69	76·09	20·81	0·83	1·40
7–3–47	19·5	80	74·13	22·04	1·46	1·76
21–3–47	19·5	80	73·84	19·64	1·74	2·11
26–6–47	13·3	20	75·88	21·98	0·69	1·54
14–7–47	13·2	18	77·09	20·48	1·00	1·67
4–8–47	19·8	88	73·14	20·62	3·87	1·57
12–8–47	13·5	26	76·14	18·84	1·68	1·54
1–9–47	18·5	69	75·60	20·34	2·55	1·40
16–9–47	17·5	62	75·23	19·73	2·32	1·28
1–10–47	18·5	70	75·01	20·29	3·20	1·35
16–10–47	19·3	82	72·69	20·07	5·93	1·48
3–11–47	21·5	115	68·68	21·77	8·50	1·33
25–11–47	21·5	90	73·99	21·83	3·34	1·56
2–12–47	19·0	75	72·95	21·80	4·54	1·48
17–12–47	20·2	87	74·63	20·09	3·98	1·50
7–1–48	20·2	87	74·42	20·56	3·05	1·64
16–1–48	21·5	104	73·58	19·60	4·71	1·88
11–2–48	22·3	131	72·97	22·04	3·76	1·42
24–2–48	22·8	124	73·26	21·27	2·86	1·50
3–3–48	21·4	116	70·86	20·96	6·44	1·45
13–3–48	22·4	128	73·14	20·57	4·19	1·66
16–4–48	22·5	141	72·19	18·47	3·98	1·57
3–5–48	22·8	123	75·04	19·21	4·00	1·61
18–5–48	22·3	124	72·92	22·52	2·26	1·67
2–7–48	23·8	162	73·28	20·71	5·29	1·72
3–8–48	15·8	39	76·76	18·27	4·04	1·54
23–8–48	15·0	40	75·11	20·32	1·91	2·02
8–9–48	17·8	65	71·57	21·37	2·07	1·71
22–9–48	16·8	51	74·84	20·90	1·38	1·73
16–10–48	20·2	92	72·21	21·81	3·72	1·71
25–10–48	20·1	92	71·94	..	3·51	..
8–11–48	20·8	107	67·28	22·11	7·86	1·91
26–11–48	17·1	55	72·47	21·09	3·39	1·93
7–12–48	21·0	107	72·01	21·28	4·07	1·82
16–12–48	20·8	102	72·81	..	4·47	..
29–12–48	20·9	98	69·92	..	2·29	..
12–1–49	20·9	91	74·81	21·52	1·77	2·04
25–1–49	19·9	79	71·25	..	3·05	..
7–2–49	20·8	87	72·81	22·70	2·50	1·72
23–2–49	21·4	89	75·93	21·72	0·94	1·74
4–3–49	21·2	86	75·16	22·99	1·12	1·93
24–3–49	21·3	103	74·71	..	2·45	..
28–3–49	21·3	109	72·75	..	2·16	..
12–4–49	21·7	114	74·61	21·49	4·33	1·73
26–4–49	..	..	75·50	..	1·93	..
7–5–49	21·8	121	73·86	22·70	2·14	1·94
16–5–49	22·3	124	73·23	22·02	3·13	2·10

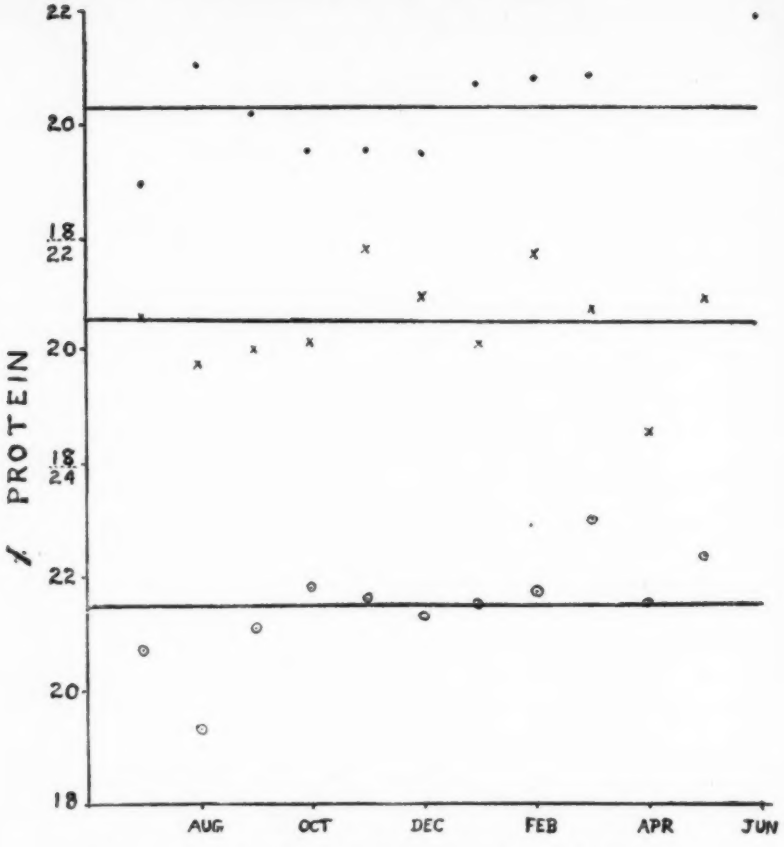

FIG. 1. Prote in Relationship in Mackeral Muscle for Three Years

. 1946–47
× ..... 1947–'48
⊙ ..... 1948–'49

15 numbers of the fish were taken on each occasion for analysis. The time between the actual catching and the analysis was about 6 hours. After recording the length and weight of each fish, analysis for the chemical constituents was conducted on the flesh. The analyses were done once in a fortnight subject to limitations of availability of the fish.

Method of Sampling.—Since the study was on the seasonal variation of the chemical composition of the flesh of mackerels, the method of sampling the flesh of the fish was similar to that given in the methods of analysis by the Association of Official Agricultural Chemists (U.S.A., 1945). It may

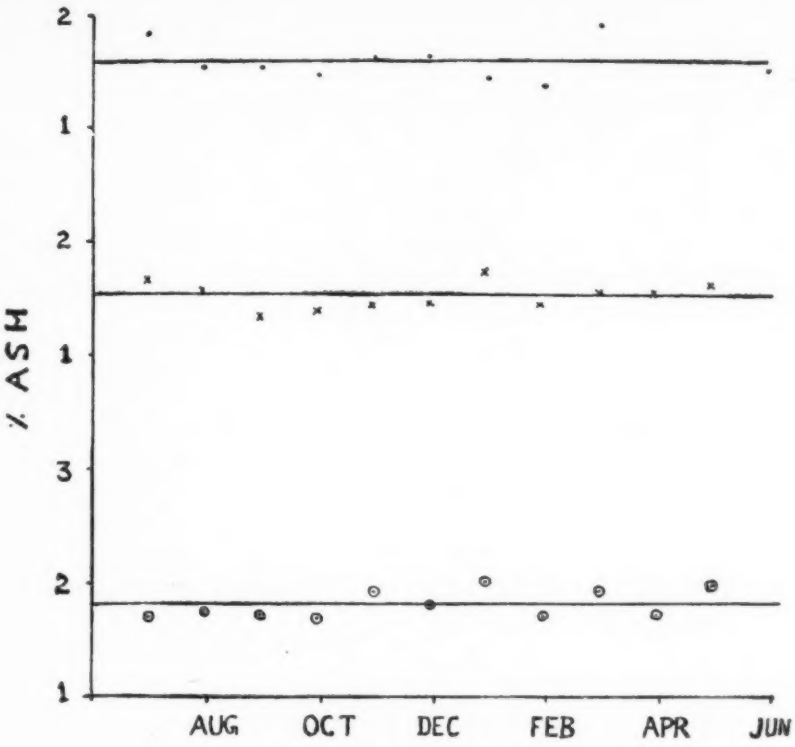

FIG. 2. Ash Variation in Mackerel Muscle.

. ..... 1946–'47
× ..... 1947–'48
⊙ ..... 1948–'49

be mentioned here that variations in the composition of the flesh in different parts of the body of the fish and in the two sexes are likely to occur, but this aspect of the question was not examined in detail.

Estimation.—The Chemical Constituents, *viz.*, water, protein, fat and ash were estimated in the minced muscle as detailed by one of us (Chari, 1948). The histological method of assay of the oil content in fish flesh (Wilson, 1939) and of the visual judgment method of determination of fat in the body cavity (Hjort, 1914; Bjerken, 1917) to demonstrate the ontogenetic variations in fat of fish are well known. As these methods are intended to indicate only the degree of fatness of the fish but not the chemical correlation, they were not followed in the estimation of fat in the flesh. Fat

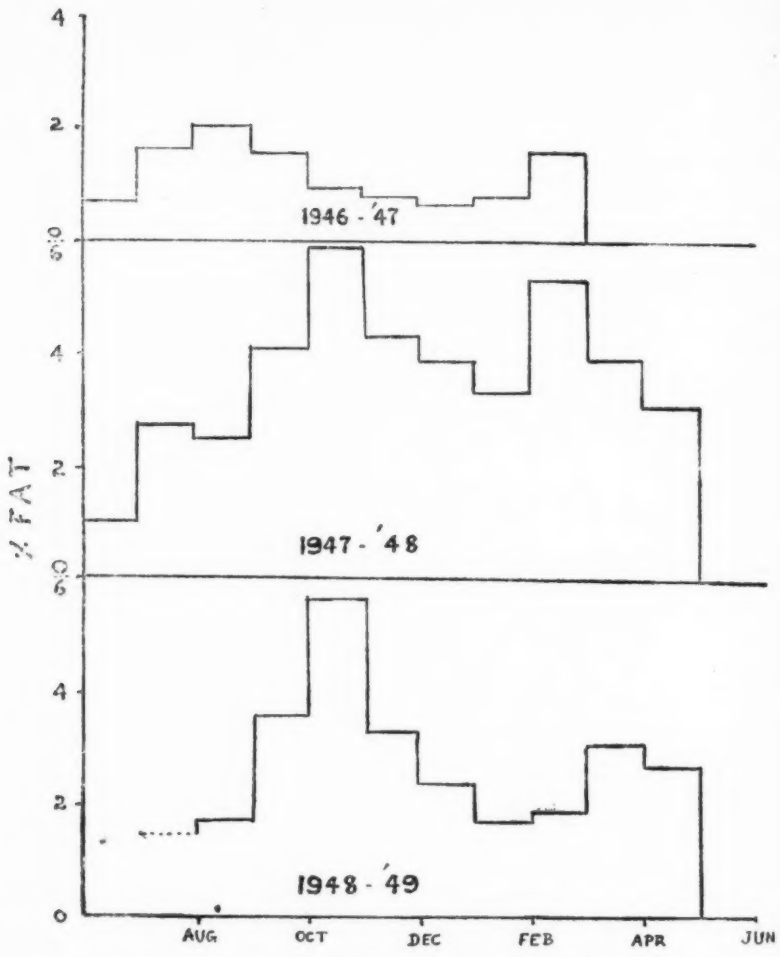

FIG. 3. Fat Variation in Mackerel Muscle for 3 Years

extraction was done throughout on the dried muscle tissue in a Soxhlet extraction apparatus with ethyl ether for 12 to 16 hours.

RESULTS

Results of the analysis on various dates during the three years are given in a consolidated table (Table I). The graphs, Figs. 1 to 4, depict the various data in a perspective form.

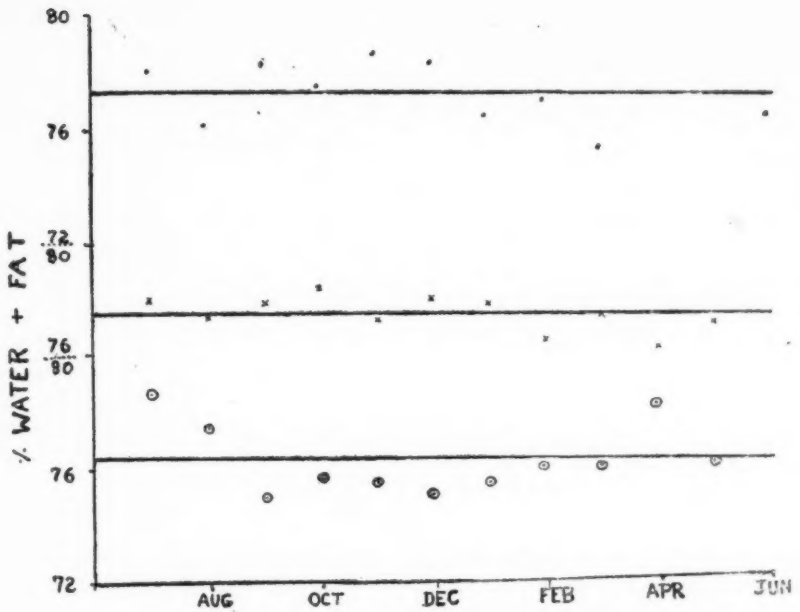

FIG. 4. Relationship of Water Plus Fat in Mackerel Muscle for 3 Years

. ..... 1946–'47
× ..... 1947–'48
⊙ ..... 1948–'49

Graph No. 1 gives the monthly protein relationship; No. 2 the ash variation; No. 3 the fat variation and No. 4 the constancy of the percentage of water and fat for a period of 3 years.

DISCUSSION

On a scrutiny of the Table it will be observed that there is a direct arithmetical relationship between the length and weight of the mackerel, which is to be expected. Generally as the length increases, the weight of the fish also increases and *vice versa*.

The protein variation as seen in Graph No. 1 resembles that of the ash variation (Fig. 2) but is not so great as in the case of fat (Fig. 3). The computation of the average protein contents and of the deviations reveals that the protein content is fairly constant within the limits shown below:—

1946–47	20·27% ± 0·06 to 1·71%
1947–48	20·55% ± 0·07 to 2·08%
1948–49	21·43% ± 0·06 to 2·14%

Graph No. 2 which portrays the ash content of mackerel flesh, reveals a greater degree of constancy during the three years than that observed for the protein content, confirming Arevalo's observations (1948).

Water and fat are the only great variables which indicate a definite reciprocal relationship as may be seen in Graph No. 4 and the table. Graph No. 3 shows the seasonal variations in the fat content of the flesh of mackerel and No. 4 the general relationship of the sum of water and fat contents. The maximum fat content was 2·83% in September 1946, 8·5% in November 1947 and 7·8% in November 1948. The minimum was 0·7% in January 1947, 1·0% in July 1947 and 1·7% in July 1948. The general pattern of the variation of the fat content in the muscle of the mackerel for the two years 1947–48 and 1948–49 closely resemble each other though the graph for 1946–47 is slightly different. The graphs for the two years 1947 to 1949 show that the fat of the fish increases from September reaching a peak in November after which there is a steady fall up to February and a slight increase thereafter. Hornell and Naidu (1924) observed a sudden rise in the body fat of oil sardine in October which continued till the end of November when it began to fall rapidly. As the maximum of oil was obtained in November of 1920 and 1921, they seem to have correlated their oil peak with the planktonic abundance during the period September to December. The statistics of mackerel catches on the Malabar coast enable us to state that the fishery reaches its peak in the September to November period, and it is during this period that the fat content in the fish also attains its maximum as is brought out by Graph No. 3. Further, Chidambaram and Menon (1945) report that the peak of the general fishery of this coast coincides with the abundance of planktonic growth during September to November which is further indicated by our investigation on fat which reaches the maximum during this period. We may, therefore, state that the fat content varies directly with the amount of food available in the Sea (Lovern and Wood, 1937; Riegol, 1948).

The fact that water and fat in a fish have a reciprocal relationship and that the sum of the two is quite close to a constant value is amply proved by Graph 4 for the three years and is in conformity with the results obtained elsewhere (Milroy, 1908; Bruce, 1924; Hart, *et al.*, 1940; Stansby and Lemon, 1941; Canwyk, 1944).

A closer scrutiny of the 'fat variation' reveals monthly fluctuations. But within certain limits, a forecast can be made as to when the mackerels will be fatty or lean.

Summing up, the present paper deals only with the water, protein, fat and ash contents of mackerel and the seasonal fat variation in the flesh of

mackerels. No biological or hydrobiological data involved has been dealt with. It is proposed to deal with these with special stress on fat variation in due course in collaboration with the staff of the Madras Fisheries Marine Biological Station, West Hill. It is anticipated that the fat analyses of whole mackerel, gonads and plankton, side by side with the flesh of the fish may throw further light on the fluctuation.

Conclusion

The following general conclusions can be drawn on the seasonal variations in the chemical composition of mackerels:—

1. The weight of fish varies directly as length.

2. The tendency of ash and protein content of mackerel is to remain constant.

3. Water and fat are the components of fish which are subject to seasonal variation and have a reciprocal relationship.

4. The fat contents in mackerel rise to a maximum between September to November and fall thereafter gradually.

5. The average composition as revealed by analysis in the years 1947 to 1949 is shown below:—

Water	..	..	73·45%
Protein	..	..	20·95%
Fat	..	..	3·29%
Ash	..	..	1·66%

Acknowledgements

Our thanks are due to Mr. K. Chidambaram, Assistant Director of Fisheries (Marine Biology), West Hill, for his co-operation throughout the period of study and useful discussions on this subject.

References

1. A. O. A. C. .. *Methods of Analysis by Assoc. of Official Agricultural Chemists*, Sixth Edition, 1945, p. 359–64.
2. Arevalo, A. .. "Variations in the chemical compositions of the Sawel (*Trachurus traehurus*)," *Chem. Abs.*, 1949, **43**, 3115.
3. Atwater, W. O. (1888) .. *Rep. U.S. Comm. Fish.*, 1892, **16**, 679–868.
4. Bjerkan, P. .. "Age, maturity and quality of North sea herring, during the years 1910–13," *Rep. Norweg. Fish. Mar. Invest.*, 1917, **3**, No. 1, 1–119.
5. Bruce, J. R. .. "Changes in the chemical composition of the tissues of the herring in relation to age and maturity," *Biochem. J.*, 1924, **18**, 469–85.

6. Bull, H. O. .. "The relationship between state of maturity and chemical composition of the whiting," *J. Mar. Biol. Assoc.*, 1928, **15**, 207–18.

7. Canwyk, G. F. .. "South African Fish Products. VIII. Composition of the flesh of Cape fishes," *J. Soc. Chem. Ind.*, 1944, **63**, 367–71.

8. Chari, S. T. .. "Nutritive value of some of the West Coast marine food fishes of the Madras Province," *Ind. J. Med. Res.*, 1948, **3**, 253–59.

9. Chidambaram, K. and Devidas Menon, M. "The correlation of the West Coast fisheries with plankton and certain oceanographical factors," *Proc. Ind. Acad. Sci.*, 1945, **22**, 355–67.

10. Devanesan, D. W. .. "A brief investigation into the causes of the fluctuation of the Annual fishery of the Oil Sardine of Malabar (*Sardinella longiceps*); determination of its age and an account of the discovery of its eggs and spawning," *Report No. I of* 1942, *Madras Fish. Bull.*, 1943, **28**, 1–38.

11. El Saby, M. K. .. "A chemical study of the Egyptian Sardinella," *Notes and Memoirs of the Ministry of Comm. & Ind., Egypt*, 1937, No. 29, 1–25.

12. Hjort, J. .. "Fluctuations in the great fisheries of Northern Europe," *Rapp. Proc. Verb. Int. Cons. Explor. Mer.*, 1914, **20**, 1–228.

13. Hoar, W. S. .. "The weight-length relationship of the Atlantic Salmon," *J. Fish. Res. Bd. Can.*, 1940, **4**, 441–60.

14. Hart, J. L., Tester, A. L., Beall, D. and Tully, J. P. "Proximate analysis of British Columbia herring in relation to season and condition factor," *ibid.*, 1940, **4**, 478–90.

15. Hornell, J. and Ramaswami Naidu, M. "A contribution to the life-history of the Indian sardine," *Madras Fish. Bull.*, 1924, No. 17, 129–97.

16. Johnstone, J. .. "The fat content of Irish Sea herring," *Proc. and Trans. Liverpool Biol. Soc.*, 1915, **29**, 216–23.

17. Lovern, J. A. and Wood, H. "Variation in the chemical composition of herring," *J. Mar. Biol. Assoc.*, 1937, **32**, 281–93.

18. Maria Jesus (1944) .. "Rapid determination of water and fat in fish," *Chem. Abs.*, 1946, **40**, 2903.

19. Milroy, T. H. .. *Biochem. J.*, 1908, **3**, 366–90.

20. Osaria de Cerqueira .. "Method for determination of Oil," *Chimie and Industrie*, 1946, **56**, 259.

21. Oya, T., Usui, Y. and Sukegawa, T. "On the fat content of *Sardinia melanosticta*," *Bull. of the Jap. Soc. of Sci. Fish.*, 1937, **5**, 308–10.

22. Riegol, A. F. .. "Seasonal variations in the nutritive value of the Sardine (*Sardinia pilchardus*)," *Chem. Abs.*, 1948, **43**, 3115.

23. Stansby, M. E. and Lemon, J. M. "Studies on the handling of fresh mackerels (*Scomber scombrus*)," *U.S. Fish and Wildlife Ser. Res. Rep.*, 1941, No. 1, p. 1–46.

24. ———— .. "Quantitative determination of oil in fish flesh," *Ind. Eng. Chem.* (Anal. Edn.), 1937, **9**, 341–43.

25. Wilson, D. P. .. "Seasonal variations in the fat contents of the flounder," *J. Mar. Biol. Assoc.*, 1939, **23**, 361–79.

ROLE OF TRACE ELEMENTS IN CONTROL OF ROOT-INFECTING FUNGI*

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Received August 5, 1950

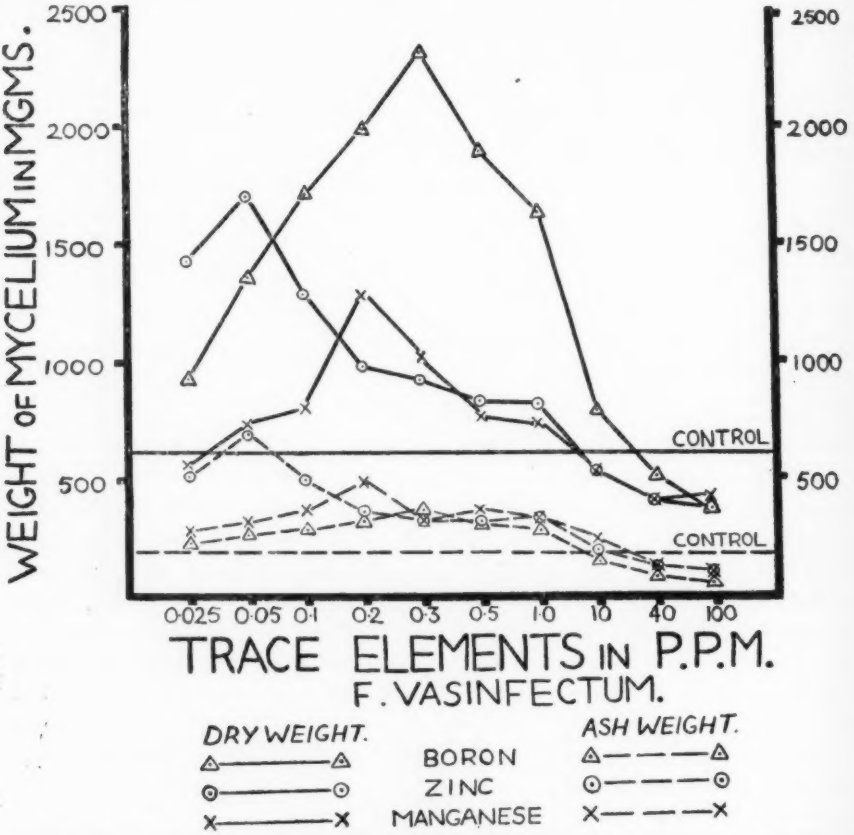
The role of trace elements in the metabolic processes of plants have been found to be of fundamental significance and to-day these trace elements are receiving a major share of attention from Soil Scientists, Plant Physiologists and Pathologists. Earlier work by the French School of workers—Javillier (1908) on zinc, Agulhon (1910) on boron and Bertrand (1927) on manganese—was mainly responsible for awakening interest on the effect of heavy metals on plant growth. Later work of Mazé (1914, 1915 and 1919), McHargue (1919; 1927), Warington (1923; 1926), Brenchley (1914; 1936), Brenchley and Warington (1927), Sommer and Lipman (1926) furnished very convincing data to prove the essentiality of many of these trace elements for proper growth and development of green plants.

Working on similar lines from the fungal point of view the essential nature of trace elements for the growth in pure cultures of fungi, in general, has been established by Roberg (1928, 1931), Metz (1930), Wolf and Emmerie (1930), McHargue and Calfee (1931), Porges (1932), Niethammer (1938), Rogers (1938), Blank (1941) and Ezekiel (1945). Recently, comprehensive reviews of the exact status of trace elements in relation to fungal growth and metabolism have been written by Steinberg (1939; 1950), Foster (1939), Stiles (1946) and Perlman (1949).

Nevertheless, the first practical evidence of control of soil-borne fungal diseases of wheat caused by *Helminthosporium sativum*, *Curvularia ramosa*, *Fusarium culmorum* and *Rhizoctonia solani* by the application of zinc as zinc sulphate at rates of 15 to 30 lb. per acre came more recently from Millikan (1938). An earlier review by Sadasivan (1950) summarises the work done in this laboratory on micro- and macro-nutrient elements in relation to soil-borne fungal diseases of plants. The present review, however, combines observations on the role of some trace elements on the growth in pure culture of three species of the genus *Fusarium* (causal agents of wilts of certain crop plants) and also their saprophytic and parasitic activity in trace element-amended "wilt-sick" soils.

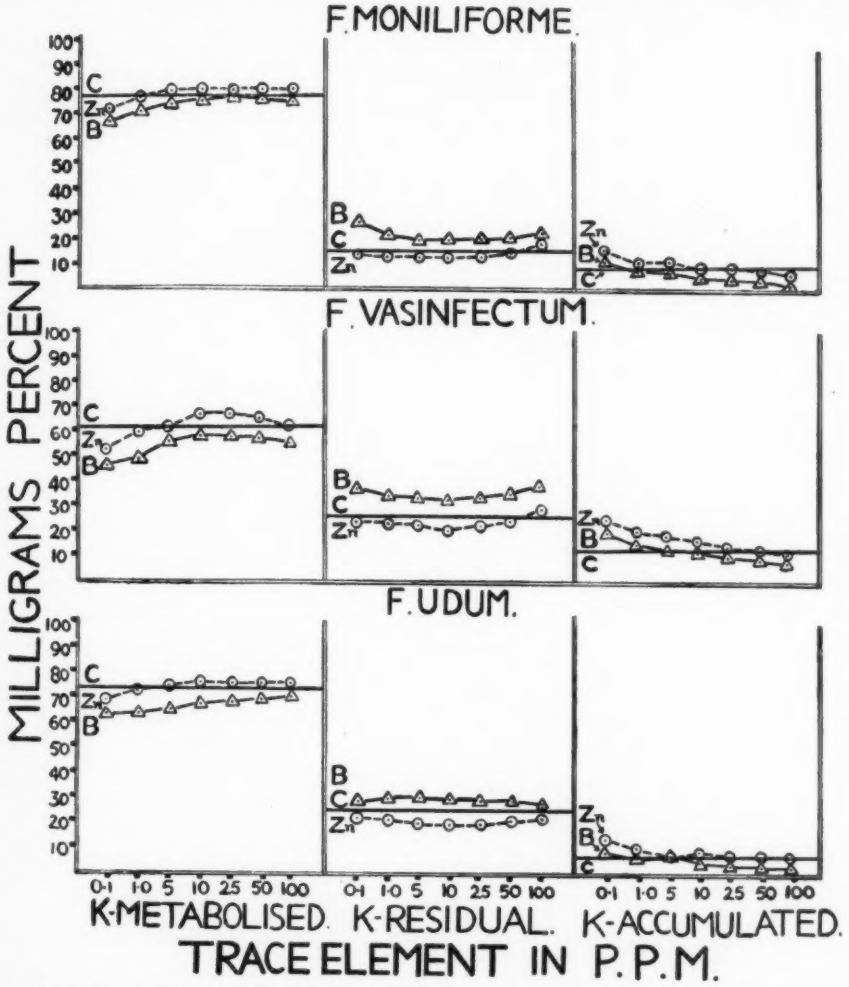
* Paper presented at the VII International Botanical Congress, Sweden, 1950.

Recent work in this laboratory by Yogeswari (1948) using three soil Fusaria, *F. vasinfectum*, cotton wilt pathogen, *F. udum* the causal agent of wilt in *Cajanus cajan* and *F. moniliforme* infecting paddy showed that al three fungi responded well to various concentrations of boron, zinc and manganese added to Richard's liquid medium. Optimum levels for growth were low being 0·3 p.p.m. of boron, 0·05 p.p.m. of zinc and 0·2 p.p.m. of manganese for *F. vasinfectum*, 0·5 p.p.m. of boron, zinc and manganese for *F. udum* and 0·3 p.p.m. of boron, zinc and managanese for *F. moniliforme*. Higher concentrations of these trace elements, however, were not toxic. The point of importance noticed was that all three species of the fungus yielded heaviest dry weights in plus boron nutrient media but highest ash weights were recorded in plus zinc nutrient media (*vide* Text-Fig. 1).



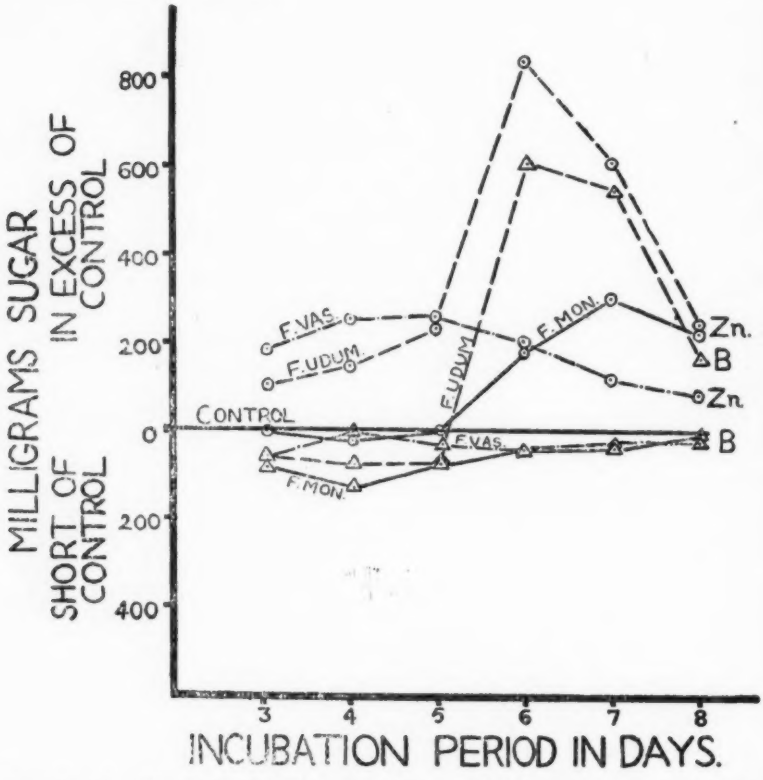
TEXT-FIG. 1. Showing increased dry and ash weights of *F. vasinfectum* in the presence of trace-elements in Richard's nutrient medium over its control.

Yogeswari attributed the increased ash weight in the presence of zinc to accumulation in fungal matrix of potassium and magnesium from the liquid culture substrate and that selective absorption of inorganic salts takes place to a greater extent in the presence of zinc but not boron. However, dry weight of the mycelial mats increased in the presence of boron only, pre-



TEXT-FIG. 2. Showing quantitatively potassium metabolised [(?) this indicates K-not accountable in residual culture solutions and fungal ash], residual potassium and potassium accumulated by three species of *Fusarium* in the presence of Boron and Zinc. (C = control ; B = + Boron ; Zn = + Zinc.)

sumably due to accumulation of carbohydrates. This became obvious since on incineration lower ash weights were recorded in the case of boron over zinc. Yogeswari (1950) clarified these two points by further experimentation (Text-Figs. 2 and 3).



TEXT-FIG. 3. Showing quantitatively variations in residual sugar (as sucrose) left behind by three species of *Fusarium* in the presence of Boron and Zinc.

The results in Text-Fig. 2 show increased metabolism and accumulation of potassium in plus zinc cultures over plus boron cultures and the control. This fact is corroborated by increased residual potassium in plus boron cultures whereas plus zinc cultures show the least amount of residual potassium. Comparing Text-Figs. 2 and 3 it is obvious that sucrose consumption from identical substrata is quantitatively more in plus boron cultures than plus zinc cultures but the amount of sucrose utilised by the fungi is greater in plus zinc cultures than plus boron resulting in increased accumulation of carbon in plus boron cultures over plus zinc and control

cultures. It may be mentioned that increased respiratory activity in *Aspergillus niger* in the presence of zinc is already known (Buromsky, 1913; Wassiljew, 1935) and the above results confirm once again quantitatively such behaviour in the three soil-borne species of the genus *Fusarium*.

The work of Yogeswari therefore brings into relief two new factors in *Fusarium* metabolism, namely, that the addition of zinc to culture media of known C/N ratio increases the ash weight of the fungi due to increased accumulation of inorganic salts, particularly potassium. On the other hand, the addition of boron brings about increased accumulation of carbon in the fungal matrix. The application of these results in further study of soil-borne pathogenic Fusaria is obvious since the increased presence of certain of these trace elements in soils where *soil-inhabitant* group of Fusaria persist would bring about a rapid consumption of available soil potassium from arable soils. Indeed, several aspects of the exact function of trace elements like growth response of plants, protection of wilt in plants, saprophytic activity and colonisation on plant debris in soils have been undertaken in this laboratory by Sarojini (1950) and Sulochana (1950).

Sarojini's results brought to the forefront an important aspect of trace element amendment of " wilt-sick " soils, *viz.*, the inhibition of saprophytic capabilities of *Fusarium* infection present in gram " wilt-sick " soils when red gram stem pieces were buried in such soils (Tables I and II).

TABLE I

Showing percentage colonisation of Fusarium *on Red Gram stem pieces in micro-nutrient amended soils*

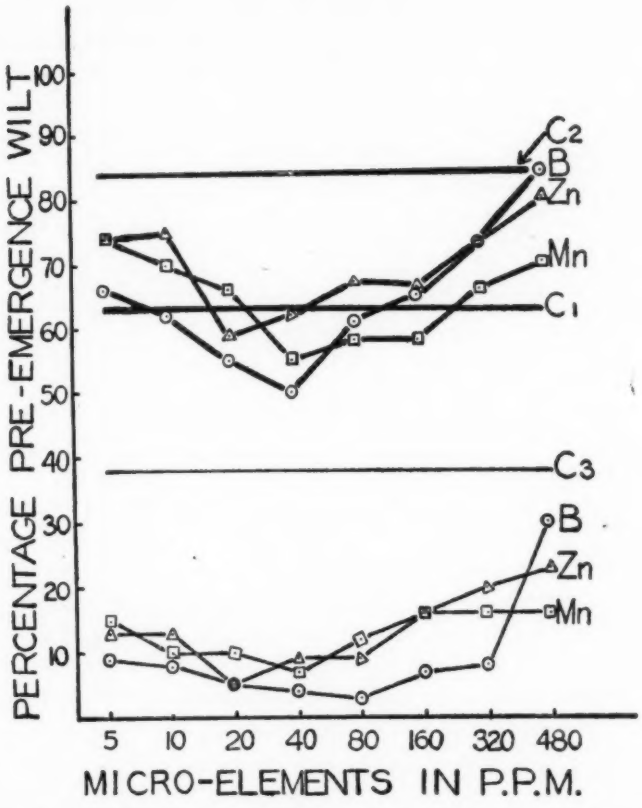
Micro-element	Levels in p.p.m.	Period of incubation in months			
		1	2	3	4
Boron ..	20	20	8	8	34
	40	16	25	66	84
	80	4	20	44	76
Manganese ..	20	26	35	77	92
	40	4	20	76	88
	80	16	37	84	92
Zinc ..	20	20	34	13	..
	40	12	8	..	..
	80	12	7	..	..
Control ..		100	100	100	100

TABLE II.

Showing percentage survival (viability) of Fusarium udum *on Red Gram stem pieces in micro-nutrient amended soils*

Micro-element	Levels in p.p.m.	Period of incubation in weeks							
		1	2	3	4	5	6	7	8
Boron	20	96	84	72	56	32	16	12	4
	40	96	80	76	56	36	12	..	..
	80	90	80	76	62	32	8	..	..
Manganese ..	20	90	84	76	64	36	20	12	12
	40	92	84	76	64	40	24	16	4
	80	90	80	72	60	40	20	16	8
Zinc ..	20	75	57	38	12	4	..	..	..
	40	76	48	32	8	..	..	..	..
	80	75	47	30	6	..	..	..	..
Control ..		100	100	96	88	84	80	76	76

It was observed that addition of boron and manganese brought about a decreased colonisation at the end of the first month and towards the end of four months, except in the case of 20 p.p.m. boron, the others showed a high percentage colonisation although, still less than the control. Zinc, however, seemed to have an adverse effect on colonisation of *Fusarium*, and there was no colonisation at the end of 3rd or 4th months in all the strengths of micro-elements used, although, at 20 p.p.m. there was some colonisation at the end of the 3rd month. Similarly Sarojini's results in the percentage viability of *F. udum* when buried in garden compost soil in the form of laboratory colonised red gram stem bits are of considerable interest in that the most rapid disappearance of *F. udum* was brought about with the addition of zinc nutrient solutions, the fungus being exterminated from the colonised stubble within 6 weeks' burial in the soil as against the high percentage viability maintained by the fungus in the control soils. However, the most interesting results were obtained in the matter of percentage pre-emergence wilt of *Cajanus* in " wilt-sick " soils with the addition of micro-nutrient elements boron, manganese and zinc at various levels in pathogenicity trials (Text-Fig. 4). Broadly speaking, micro-nutrient treated series on the whole yielded better results than the control, by lowering the disease index to an appreciable extent and at the same time promoting plant growth (Table III).



TEXT-FIG. 4. Showing percentage pre-emergence wilt of *Cajanus cajan* in micro-element amended, wilt-sick soil caused by *F. udum*. Top five bold lines represent micro-element amended November soils, bottom thin lines represent amended March soils along with their controls C_1, C_2 and C_3.

C_1 : bare November soil ;
C_2 : November soil *plus F. udum* inoculum ;
C_3 : bare March soil.

Comparing the results presented in Text-Fig. 4 and Table III it is apparent that, of the treatments tried, manganese afforded maximum protection from wilt to the red gram plant followed by zinc and boron in the decreasing order of efficiency. It is interesting to note that at the levels used manganese and zinc did not produce any apparent toxic symptoms even with the highest dosage used in the series. Indeed, the earliest flowering was noticed with the combination of the three elements boron, manganese and zinc although, individually too, they were as good as, if not slightly better than,

TABLE III

Showing growth response of Red Gram (Cajanus cajan) *to micro-nutrient amendments in soils*

Micro-element	Levels in p.p.m.	Height in inches at 60 days	Flowering in days after sowing	Dry weight of seeds in gm.
Boron ..	20	24	143–147	6·526
	40	28	146–155	6·772
	80	17	149–178	6·360
Manganese ..	20	36	144–145	6·923
	40	29	138–144	7·054
	80	21	138–144	7·109
Zinc ..	20	29	148–150	6·691
	40	27	146–151	6·819
	80	25	141–147	6·674
Boron+Manganese+Zinc ..	20	36	130–132	6·867
	40	41	120–123	6·876
	80	24	135–138	7·262
Control ..		16	149–155	6·564

the control. Dry weight of seeds produced in the combination of elements was also better than individual elements and the control. These investigations make it abundantly clear that the trace elements boron, zinc and manganese have an important role to play in the control of wilt of plants.

Sulochana (1950), working on other aspects of micro-nutrient elements and the behaviour of *F. vasinfectum* in soil, considerably expanded the list of micro-elements of Sarojini and studied the effect of colonisation and survival of the fungus on buried root-pieces (Table IV) and also the effect of micro-nutrient elements in pure culture on sporulation of *F. vasinfectum* (Table V).

Of the micro-nutrient elements employed in the tests, lithium and molybdenum showed maximum inhibition in micro- and macro-conidial sporulation by *F. vasinfectum*. The practical application of this aspect, *viz.*, the anti-sporulating effect produced by trace elements on soil fungi is not properly assessable but it appears to be an important aspect worthy of consideration in future studies on soil fungi. As regards the saprophytic activity of *F. vasinfectum* in soils, of the eight elements tried boron inhibits the saprophytic activity of *F. vasinfectum* by reducing both the percentage colonisation on root-pieces as well as the percentage survival in colonised root-pieces at 80 p.p.m. concentration over a period of 24 weeks' incubation.

TABLE IV

Showing percentage colonisation of Fusarium *and survival of* Fusarium vasinfectum *on cotton stubbles in micro-nutrient amended "wilt-sick" soil at* 80 *p.p.m. concentration*

Micro-element	Colonisation per cent.				Survival per cent.			
	Incubation period in weeks				Incubation period in weeks			
	4	8	16	24	4	8	16	24
Manganese ..	100	80	56	56	92	72	64	40
Molybdenum ..	84	72	32	32	100	100	100	100
Cobalt ..	80	60	34	30	100	100	100	100
Zinc ..	76	44	24	20	88	64	32	20
Aluminium ..	52	40	20	8	100	100	100	100
Boron ..	52	48	20	12	80	60	28	12
Lithium ..	56	40	20	12	80	64	32	16
Nickel ..	52	36	24	8	100	100	100	100
Control ..	100	100	100	100	100	100	100	100

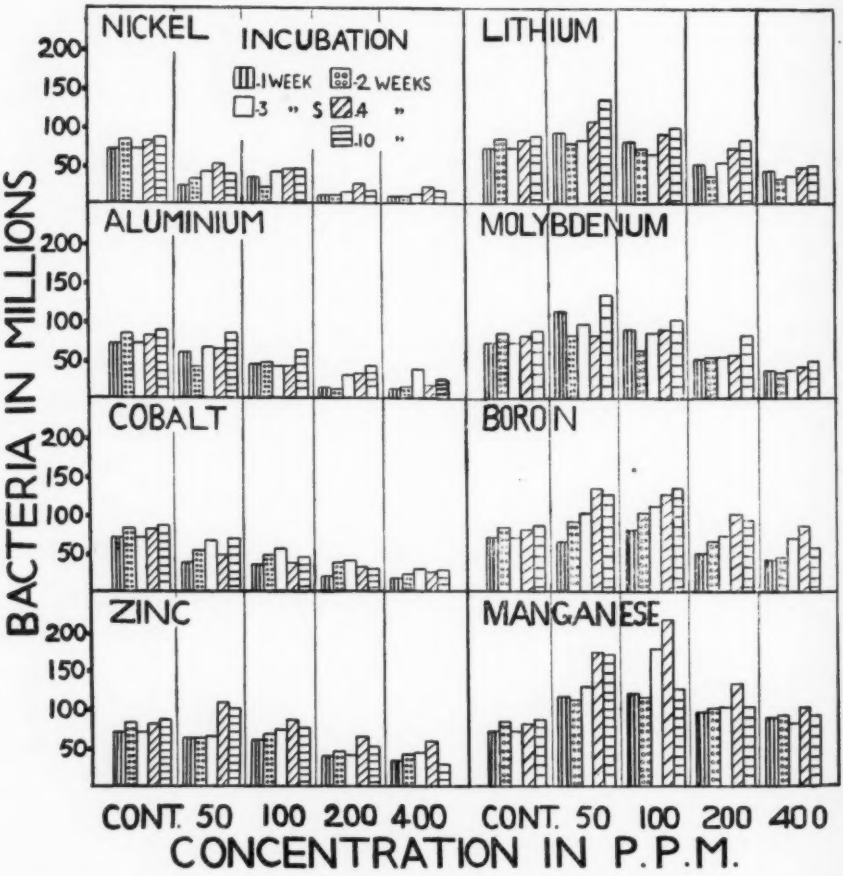
TABLE V

Showing effect of micro-nutrient elements in pure culture on sporulation of Fusarium vasinfectum

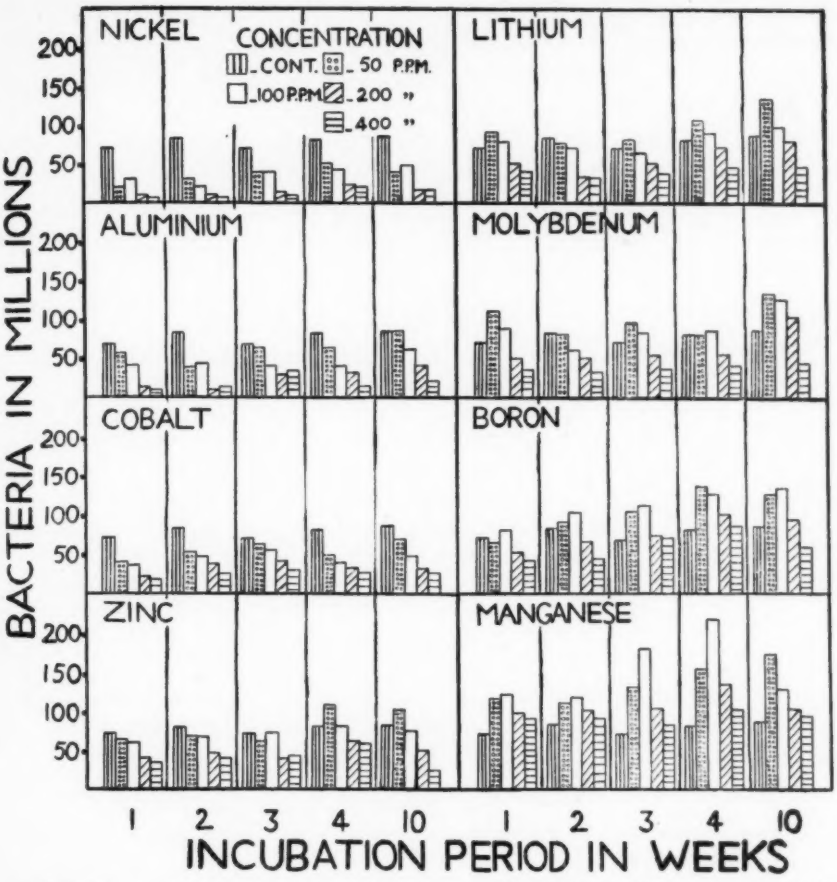
Micro-element	Micro- and Macro-conidial numbers in millions					
	Micro-elements in p.p.m.					
	50	100	250	500	750	1000
Manganese ..	11·20	8·60	6·30	5·60	5·23	5·20
Nickel ..	7·33	4·10	0·53	..	..	..
Boron ..	6·60	5·80	5·13	4·43	3·83	3·06
Molybdenum * ..	6·30	6·30	5·80	1·33	0·13	0·065
Molybdenum† ..	2·76	1·90	0·53	..	..	..
Zinc ..	6·00	4·10	2·50	2·05	1·60	0·09
Vanadium ..	5·40	5·20	5·13	2·63	2·03	1·40
Aluminium ..	4·20	2·70	1·70	1·56	1·30	0·065
Lithium ..	2·48	0·003	..	..	..	..
Uranium ..	4·45	4·31	3·67	3·30	2·50	2·30
Thorium ..	7·34	6·85	6·24	5·50	4·60	3·70
Cobalt ..	4·88	4·20	3·75	2·60	1·03	0·65
Rubidium ..	9·50	6·50	6·35	5·60	5·20	5·01
Cadmium ..	1·60	0·95	0·80	0·65	0·02	0·005
Control ..	12·08	..	..	..	..	..

* Molybdenum trioxide. † Ammonium molybdate.

Sulochana's work also showed that quantitatively the bacterial population of the various micro-nutrient element amended soils showed varying degrees of response, thus, large-scale statistical interpretation of her results clearly indicated that the maximum increase was brought about by the addition of manganese and that the eight elements tried could be classified in the descending order of efficiency as Mn > B > Mo > Li > Zn > Co > Al > Ni. This important result showed itself clearly when the data was analysed by studying the interaction between the concentration of micro-element and



TEXT-FIG. 5. Showing increase or decrease in bacterial numbers over control in micro-nutrient element amended soils. Concentration of micro-nutrient element amendment remain constant, while incubation varies.



TEXT-FIG. 6. Snowing increase or decrease in bacterial numbers over control in micro-nutrient element amended soils. Incubation period remains constant, while concentration of micro-nutrient element amendment varies.

incubation and *vice versa*. Thus, in both cases, when concentration of the micro-element amendment was constant with the period of incubation varying and also when the period of incubation was constant and the concentration of micro-element varying the same descending order of efficiency of the trace elements in increasing bacterial population with manganese effecting maximum increase and nickel the least was noticed (Text-Figs. 5 and 6). Nevertheless, it must be mentioned that from the point of view of effective control of colonisation and saprophytic activity of *F. vasinfectum* the addition of boron seems to be more beneficial than manganese and the

anomaly is explainable since boron not only stimulates increase in bacterial numbers, though not to the extent of manganese, yet has the additional advantage over manganese in being able to suppress sporulation of *F. vasinfectum* (Table V).

The effectiveness of boron, therefore, in reducing the percentage survival and percentage colonisation of *F. vasinfectum*, considerably over the control, is attributable to its ability to inhibit sporulation as well as to increase microbial antagonism in soil, which dual function is not attributable to the other elements tried.

Literature Cited

Agulhon, H. .. "Emploi du bore comme engraise catalytique," *C. R. Acad. Sci., Paris*, 1910, **150**, 288–91.

Bertrand, G. .. Quoted from *Inorganic Plant Poisons and Stimulants*, by W. E. Brenchley, Cambridge University Press, 1927, Vol. II.

Blank, L. M. .. "Response of *Phymatotrichum omnivorum* to certain trace-elements," *J. agric. Res.*, 1941, **62**, 129–59.

Brenchley, W. E. .. "The action of certain compounds of Zinc, Arsenic and Boron on the growth of plants," *Ann. Bot.*, 1914, **28**, 283–301.

———— .. "The essential nature of certain minor elements for plant nutrition," *Bot. Rev.*, 1936, **2**, 173–96.

——— and Warington, K. .. "The role of boron in the growth of plants," *Ann. Bot.*, 1927, **41**, 167–87.

Buromsky, I. .. "Die Salze Zn, Mg und Ca, K und Na und ihr Einfluss auf die Entwicklung von *Aspergillus niger*," *Zentrbl. Bakt.*, 1913, II, **36**, 54–66.

Ezekiel, W. N. .. "Synthetic culture media for the root-rot fungus *Phymatotrichum omnivorum*," *Phytopath.*, 1945, **35**, 159–61.

Foster, J. W. .. "The heavy metal nutrition of fungi," *Bot. Rev.*, 1939, **5**, 207–39.

Javillier, E. .. "Le zinc chez les plantes. Recherches sur sa présence et son rôle," *Ann. Inst. Pasteur.*, 1908, **22**, 720–25.

Mazé, P. .. "Influences respectives des élémentes de la solution minérale sur le développment due maïs," *ibid.*, 1914, **28**, 21–68.

———— .. "Determination des élémentes minér aux rares necessaires an développment du maïs," *Compt. rend. Acad. Sci., Paris*, 1915, **160**, 211–14.

———— .. "Recherche d'une solution purement minérale capable d'assurer l'evolution complète du maïs cultivé à l'abri des microbes," *Ann. Inst. Pasteur.*, 1919, **33**, 139–73.

McHargue, J. S. .. "The effect of manganese on the growth of wheat: A source of manganese for agricultural purposes," *Ind. Eng. Chem.*, 1919, **11**, 332–35.

McHargue, J. S. .. "Significance of the occurrence of manganese, copper, zinc, nickel and cobalt in Kentucky Blue grass," *ibid.*, 1927, **19**, No. 2, 272–76.

——— and Calfee, R. K. .. "Effect of Mn, Cu, Zn on growth and metabolism of *Aspergillus flavus* and *Rhizopus nigricans*," *Bot. Gaz.* 1931, **91**, 183–93.

Metz, O. .. "Über Wachstum und Farbstoffbildung einiger Pilze unter dem Einfluss von Eisen, Zinc und Kupfer," *Arch. Mikrobiol.*, 1930, **1**, 197–251.

Millikan, C. R. .. "A preliminary note on the relation of zinc to disease in cereals," *J. Dept. Agric. Vict.*, 1938, **36**, 409–16.

Niethammer, A. .. "Wachstumversuche mit mikroscopischen Bodenpilze," *Arch. Mikrobiol.*, 1938, **9**, 23–30.

Perlman, D. .. "Effects of minor-elements on the physiology of fungi," *Bot. Rev.*, 1949, **15**, 195–220.

Porges, N. .. "Chemical composition of *Aspergillus niger* as modified by zinc sulphate," *Bot. Gaz.*, 1932, **94**, 197–205.

Roberg, M. .. "Über die Wirkung von Eisen-, Zink-, und Kupfersalzen auf *Aspergillen*," *Zentrbl. Bakt.*, 1928, **74**, 335–71.

——— .. "Weitere Untersuchungen über die Bedeutung des Zinks für *Aspergillus niger*," *ibid.*, 1931, **84**, 196–230.

Rogers, C. H. .. "Growth of *Phymatotrichum omnivorum* in solutions with varying amounts of certain mineral elements," *Amer. Jour. Bot.*, 1938, **25**, 621–24.

Sadasivan, T. S. .. "Some aspects of soil-borne fungal diseases of plants," "*The Palaeobotanist*," *Birbal Sahni Memorial Volume*, 1950.

Sarojini, T. S. .. "Soil conditions and root diseases. I. Micro-nutrient element and disease development by *Fusarium udum* on red gram [*Cajanus cajan* (Linn.) Millsp.]," *Jour. Madras Univ.*, 1950, Sect. B, **19**, 1–31.

Sommer, A. L. and Lipman, C. B. "Evidence on the indispensable nature of zinc and boron for higher green plants," *Plant Physiol.*, 1926, **1**, 231–49.

Steinberg, R. A. .. "Growth of fungi in synthetic nutrient solutions," *Bot. Rev.*, 1939, **5**, 327–50.

——— .. "Growth of Fungi in Synthetic Nutrient Solutions II," *ibid.*, 1950, **16**, 208–228.

Stiles, W. .. *Trace Elements in Plants and Animals*, Cambridge Univ. Press, 1946.

Sulochana, C. B. .. "Soil conditions and wilt diseases in plants: with special reference to colonisation and survival of Soil Fusaria in soils treated with micro-elements" (Thesis approved for the Degree of Master of Science, Univ. of Madras), 1950.

Warington, K. .. "The effect of boric acid and borax on the broad bean and certain other plants," *Ann. Bot.*, 1923, **37**, 629–72.

Warington, K. .. "The changes induced in the anatomical structure of *Vicia faba* by the absence of boron from the nutrient solution," *ibid.*, 1926, **40**, 27–42.

Wassiljew, G. .. "Über die Einwirkung von Zink auf den Stoffwechsel von *Aspergillus niger*," *Arch. Mikrobiol.*, 1935, **6**, 250–75.

Wolff, L. K. and Emmerie, A. .. "Über das Wachstum des *Aspergillus niger* und der Kupfergehalt des Nährbodens," *Biochem. Z.*, 1930, **228**, 443–50.

Yogeswari, L. .. "Trace element nutrition of fungi. I. The effect of Boron, Zinc and Manganese on *Fusarium* species," *Proc. Indian Acad. Sci.*, 1948, **28**, 177–201.

———— .. "Trace element nutrition of fungi with special reference to Soil *Fusarium* spp." (Thesis approved for the Degree of Doctor of Philosophy, Univ. of Madras), 1950.

ADDENDUM

Since this article went to press the author has read with interest the work of Vasudeva and Roy (*Ann. appl. Biol.*, 1950, **37** (2), 169–78). Unpublished work in this laboratory of Thankam (summarised by the present writer in a review article in "*The Palaeobotanist*," *Birbal Sahni Memorial Volume*, 1950), had already indicated the presence of strains of bacteria in *Fusarium* wilt-infected soils from cotton fields which are capable of producing antagonism and control of wilt in cotton in pot experiments. The importance of the work of Thankam indicated three functions of the antagonising bacterial cells in reducing wilt index of cotton in sterilised soils, *viz.*, (*a*) the effectiveness of the bacterial cells themselves, when added in known quantities to soils, as millions per ml. in aqueous suspensions (using the Cholodny slide technique it has also been shown that the bacterial cells bring about a very rapid loss of viability in *Fusarium* (*F. vasinfectum*) cultures both in the mycelial and sporulating forms); or (*b*) the toxins of these bacterial populations; or (*c*) the addition of both the suspensions and the toxins. These results have been amply supported by the investigations of Vasudeva and Roy referred to above although their studies have been confined to only the bacterial toxicology part of Thankam's work (Thankam demonstrated bacterial antagonisms both in pure culture and in soils). It may, however, be mentioned here that the building up of microbial antagonism to fungi in soils is a logical approach to a system of biological control of soil-borne fungal organisms and their activity. In this field of enquiry the basic work should be one of classifying soil pathogenic fungi into those that make no free spread in unsterilised soils as against those that do (this work is now in progress in this laboratory). It would then become obvious that the former category could be antagonised more effectively by new and increasing bacterial populations as well as the use of certain trace elements (heavy

metals) which, as pointed out in this article, appear to serve a useful purpose in bringing about modifications in the host-parasite relationship. Whilst agreeing largely with the broad indications given by Vasudeva and Roy on the occurrence of the disease in *Cajanus* as related to the distribution of the antagonising bacterial floras, in patches, in the field, no attempt is made here to compare the behaviour of *Fusarium vasinfectum* on cotton (in the presence of a bacterial flora) with that of *F. udum* on *Cajanus*. Nevertheless, it could be stated that several species of the genus *Fusarium*, although very susceptible to microbial antagonism, have the remarkable ability of decomposing cellulose and possibly lignin in soils for long periods. Recent work done here indicates the possible effect of trace elements on *Fusarium* longevity in soils and this new situation makes it necessary to await further investigations in this direction before getting a clearer picture of these complicated soil mycological problems.

EFFECT OF GERMINATION ON THE NUTRITIONAL QUALITIES OF SOME OF THE VEGETABLE PROTEINS

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The vegetarian group of Indian population obtains its protein requirements mainly from cereals and pulses. Many research workers have evaluated the nutritive values of these foodstuffs from time to time. B. N. Acharya, S. P. Niyogi and V. N. Patwardhan (1942) studied the effect of parching on the biological value of proteins of some cereals and pulses. These authors found that parching definitely improved the biological value of all the proteins. The foodstuffs investigated and discussed by them, and particularly the pulses (dahls) are in many parts of India germinated before they are used in cooked as well as uncooked dishes. Though some work has been done on certain cereals and pulses, to study the changes in general, during germination, the effect of germination on the nutritional qualities of proteins has not been studied systematically, so far. An attempt has been made therefore in this investigation, to study the effect of germination on the nutritional qualities of proteins in some of the pulses.

Extensive researches have been done on the role of cotton seed in nutrition, because of its high protein content. Proteins of these seeds are not used in human foods because of the poisonous nature of the 'gossypol' which is present in them. It was suggested therefore that if the cotton seed proteins are freed from this poisonous material, they should prove to be of some value in human nutrition, in these days of food shortage. A diet containing cotton seed proteins free from gossypol, was therefore prepared and its nutritional qualities were estimated.

One of the methods of assessing the nutritive value of a protein is the balance sheet method. This method though very accurate is a laborious one and also time consuming. In order to find out the nutritive value of a particular protein in a reasonably short time, very many methods are available. The chemical method which was attempted by Almquist, Stockstad and Halbrook (1935) and Robert John Evans and J. L. St. John (1945), for the investigation of the nutritive value of proteins (which they

call ' Protein Quality Index ') was adopted in this investigation also, on those pulses that were used for the biological assay. This chemical assay was undertaken in order to correlate the results obtained by the two methods and after comparing the results thus obtained, to determine whether it could be possible to do away with the laborious and time consuming balance sheet method.

The following are the scientific names and the English and Hindi equivalents of the foodstuffs investigated here.

Scientific name	English	Hindi
1. *Gossypium herbaceum*	Cotton seed	Kapashi
2. *Cicer arietinum*	Bengal gram	Chena
3. *Lens esculanta*	Lentil	Masoor

Experimental

1. *General Analysis.*—In all eight samples were prepared from the above foodstuffs and they were analysed for their protein, fat, mineral and carbohydrate contents. Cotton seed was analysed as a whole, and then it was ground to separate the kernal from the pericarp, and the kernel and the pericarp were analysed separately. The edible portion of the germinated cotton seed was also analysed. Bengal gram, green gram and lentils were analysed before and after germination.

Method of germination.—The seeds were soaked in water for about twenty-four hours, and then the water was removed and the seeds were tied in a wet muslin cloth, till the sprouts were about quarter of an inch long. The seeds were then dried at a temperature 70 to 80° C. and powdered.

Table I

The protein, fat, ash and carbohydrate contents of the different samples calculated on moisture-free basis

(Carbohydrates have been obtained by difference)

Samples	Proteins	Fats	Ash	Carbohydrates (by difference)
	%	%	%	%
1. Cotton seed (whole) ..	25·10	33·70	6·23	34·97
2. Cotton seed kernel ..	31·80	42·50	5·78	20·72
3. Cotton seed pericarp ..	12·70	1·34	1·42	84·54
4. Kernel of germinated cotton seed ..	33·00	41·10	5·25	20·65
5. Bengal gram ..	28·00	4·78	3·15	63·27
6. Germinated Bengal gram ..	26·80	4·55	3·26	65·39
7. Lentils ..	25·20	2·10	3·02	69·68
8. Germinated lentils ..	25·00	2·42	3·75	69·83

2. *Biological Method.*—The method employed was similar to that used by Chick, *et al.* (1935 *a*, 1935 *b*), Martin and Robinson (1922) and Mitchell (1924). A brief outline of the method is given below.

Six adult rats (three males and three females) were used for each experiment. Before the first experimental diet was given the rats received the nitrogen-free diet for seven days. The first three days of each period was regarded as preparatory, allowing the rats to accommodate themselves to the new diets.

The collection of urine and fæces was confined to the remaining four days of each period. The rats were given rest for four days after each experimental period was over. During this period the rats received the stock diet. The procedure for other diets was same as in nitrogen-free diet period.

The Diets.—(*a*) *Nitrogen-free diet.*—A diet practically containing no nitrogen was prepared by using starch made nitrogen free by repeated extractions with dilute hydrochloric acid, salt mixture prepared according to Osborne and Mendel (1919), shark liver oil and sugar. It was not possible to prepare the diet absolutely free from nitrogen. The variation of the nitrogen in the nitrogen-free diet was from 0·056% to 0·0846 per cent.

(*b*) *Experimental diets.*—The experimental diets were of two types, one containing proteins of ungerminated grains and the other containing proteins of germinated grains. These were prepared by adding the necessary ingredients in the desired proportions as shown in Table II. In all the cases the nitrogen of the diets was estimated by Kjeldahl's method. In the case of cotton seed, it was first made fat-free in order to remove gossypol from it.

TABLE II

Composition of the experimental diets

Each diet contained 3 gms. of shark liver oil, 4 gms. of sesame oil and 10 gms. of sugar and other constituents. The other things are shown in the table.

Diet investigated		Experimental foodstuff gms.	Starch gms.	Percentage of nitrogen on dry weight
Nitrogen-free diet ..	..	..	75·0	0·056 to 0·0846
Cotton seed kernel (ungerminated) ..	5%	10·3	64·7	0·826
Do ..	10%	20·6	54·4	1·630
Do ..	15%	30·9	44·1	2·514
Cotton seed kernel (germinated) ..	10%	22·0	53·0	1·640
Bengal gram (ungerminated) ..	10%	35·0	40·0	1·680
Bengal gram (germinated) ..	10%	39·5	35·5	1·610

(*c*) *The stock diet.*—This was a diet prepared to contain all the constituents of the food that were necessary for the growth and maintenance of the body. The protein level of the stock diet prepared was 15·01 per cent.

Composition of the stock diet:—

Wheat flour	60 gms.
Gram flour	20 gms.
Whole milk powder	5 gms.
Sweet oil	5 gms.
Meat paste	2 gms.
Sodium chloride	0·50 gms.
Calcium carbonate	1·50 gms.
Milk	60 c.c.
Sodium hydrogen phosphate ..	3·6 gms.
Marmite	4 gms.
Ascorbic acid	4 gms.

Formula used.—The formulæ used by Chick and her co-workers, which have been adopted here are as follows:—

(*a*) The relative biological value (B.V.) of a protein (P) is expressed as:—

$$100 \times \frac{\text{Body N saved}}{\text{Body N absorbed}} = 100 \left\{1 - \frac{Up - Ue}{Ip - (Fp - Fe)}\right\}$$

where I*p*, U*p* and F*p* are the daily nitrogen intake, urinary nitrogen and fæcal nitrogen respectively on a diet containing protein; and U*e* and F*e* are urinary and fæcal nitrogen on nitrogen-free diet. (F*p* − F*e*) represents the nitrogen in the fæces derived from the undigested food protein, and I*p* − (F*p* − F*e*) the true nitrogen intake on a diet containing protein.

(*b*) The relative digestibility coefficient (D.C.) of a given protein P is expressed as:—

$$100 \times \frac{\text{Food N digested}}{\text{Food N intake}} = 100 \left\{1 - \frac{Fp - Fe}{Ip}\right\}$$

(*c*) Nett protein value was calculated from the following formula:—

$$\text{Nett protein value} = \text{percentage of protein} \times \frac{\text{B.V.}}{100} \times \frac{\text{D.C.}}{100}.$$

TABLE III

Biological value and digestibility coefficient of germinated cotton seeds and Bengal gram

Particulars of the diet	Percentage of nitrogen in the diet	No. of rats taken	Nitrogen intake (average) mg.	Urinary nitrogen (average) mg.	Fæcal N (average) mg.	Food N (average) mg.	Body N (average) mg. (Saved)	Biological valu e mg.	Standard deviaton for B. V.	Digestibility coefficient	Standard deviation for D.C.
1. N.-free diet ..	0·0846	6	2·37	25·57	14·86						
Cotton seed (protein diet) ..	0·826	6	46·30	33·58	26·42	32·34	22·97	70·47	5·36	71·53	6·48
Do ..	1·630	6	94·33	47·59	46·16	70·68	45·63	64·74	4·94	77·33	1·91
Do ..	2·514	6	159·80	81·09	56·18	115·23	62·18	53·65	2·25	78·32	6·35
2. N.-Free diet ..	0·069	6	1·84	7·56	17·10						
Germinated Coton seed protein	1·680	4	111·38	45·07	40·00	87·08	49·31	55·46	1·00	78·89	2·85
3. N.-Free diet ..	0·056	6	2·64	27·50	16·75						
Bengalgram ..	1·680	6	103·82	42·23	33·91	82·25	66·07	78·13	3·70	82·12	6·55
Do (germinated) ..	1·610	6	103·70	46·09	33·12	89·85	68·54	75·62	3·33	89·41	3·82

The standard deviation (S.D.) was calculated from the formula:—

$$\text{S.D.} = \sqrt{\frac{\Sigma d^2}{n}},$$

where (d) is the deviation of each result from the arithmetic mean and (n) is the number of readings.

3. *The Chemical Method.*—The technique employed was similar to that used by H. J. Almquist, E. L. R. Stockstad and E. R. Halbrook (*loc. cit.*) and Robert John Evans and J. L. St. John (*loc. cit.*).

The method consists of chemical determination of four protein fractions. First was the fraction of nitrogen precipitated by cupric ion, second, the nitrogen not precipitated by cupric ion but precipitated by phosphotungstic acid. Third was the fraction of nitrogen not dissolved even after prolonged digestion with pepsin, and fourth was the fraction of protein soluble in hot water and precipitated by cupric ion. The scheme is very simple and is limited to the determination of those forms of nitrogen which are considered to be of influence on the nutritive value. The chemical protein quality index was calculated from these results by the following formula:—

$$\text{Protein Quality Index (P.Q.I.)} = A - (B + 0{\cdot}6\,C) + 0{\cdot}4\,D,$$

where A is the per cent. protein (calculated from the nett protein content) precipitated by copper sulphate. B is the percentage of protein not digested with pepsin. C is the percentage of protein soluble in hot water, and D is the percentage of protein precipitated by phosphotungstic acid.

The reagents used in the above protein fractionation were:—

Copper sulphate solution	.. ..	100 gms/litre.
Alum solution	.. .. ..	100 gms/litre.
Magnesium oxide suspension	.. ..	50 gms/litre.
Hydrochloric acid solution	.. ..	250 gms/litre.
Pepsin solution	.. .. ..	5 gms/litre.
Phosphotungstic acid solution	.. ..	250 gms/litre.
Acetate buffer approx. pH	.. ..	5

Preparation of the samples.—The experimental procedure may be briefly stated as follows:—

Fraction A.—A known weight of the sample weighing between 1 to 1·2 gm. which was made fat free with ether was heated on a water-bath with 80 c.c. water for 30 minutes. 5 c.c. alum reagent, 25 c.c. copper sulphate and 50 c.c. mag. oxide suspension were added and was further heated for 20 minutes. The precipitate was filtered off after allowing the mixture to cool and its nitrogen content was found out.

To the above filtrate, 10 c.c. of phosphotungstic acid solution was added after bringing its strength to about one normal with hydrochloric acid. It was allowed to stand for twenty-four hours and the nitrogen content of the precipitate was found out. This was fraction D.

For fraction B, a fresh fat-free sample was treated with 250 c.c. of water, 5 c.c. hydrochloric acid, and 25 c.c. freshly prepared pepsin solution. The mixture was allowed to stand for forty eight hours with occasional shaking. The precipitate was filtered, washed and its nitrogen content was estimated.

Fraction C was estimated from a fat free sample by boiling it with 200 c.c. water and 5 c.c. acetate buffer. The mixture was filtered hot and the nitrogen content of the filtrate was estimated after precipitating its proteins with copper sulphate as in the case of fraction A.

TABLE IV

Table showing the fractions A, B, C, D and the protein Quality Index P.Q.I. for the different foodstuffs investigated

Protein Sample	Total N in 1 gm. mg.	Fraction A %	Fraction B %	Fraction C %	Fraction D %	P.Q.I.
1. Cotton seed kernel ..	72·30	86·95	16·33	11·41	2·25	64·68
2. Do germinated	65·65	87·52	20·57	14·15	1·78	59·23
3. Bengal gram ..	43·25	86·70	7·33	14·63	0·67	70·89
4. Do germinated	36·36	95·65	11·32	21·13	0·81	66·83
5. Lentils ..	38·66	92·50	23·56	19·88	0·47	57·18
6. Do germinated ..	35·00	93·20	26·75	24·50	0·40	51·81

DISCUSSION

It may be seen from the method of estimating the protein quality index of a protein concentrate, that the protein quality index is mainly concerned with the nitrogen retained. Therefore if there is any relation existing between the chemical method and the biological method, it will be between the protein quality index and the biological value.

The following table gives the biological values and the protein quality indices of the foodstuffs studied in this investigation.

Name of the foodstuff		B.V.	P.Q.I.
Cotton seed kernel ..	5%	70·47	64·68
Do ..	10%	64·74	
Do ..	15%	53·64	
Do germinated ..	10%	55·46	59·23
Bengal gram ..	10%	78·13	70·89
Do germinated ..	10%	75·62	66·83
Lentils ..			57·18
Do germinated ..			51·81

(The percentages are levels of protein intake)

From the above results it is seen that both the biological value and the protein quality index decrease on germination and the decrease is also of the same order, and therefore we may say that there is some parallelism between the results obtained by the chemical method and the biological method though there is no definite correlation between the two methods. How far the chemical method would be applicable in general for the determination of the nutritive value of the proteins is difficult to understand at the moment. The aspect has been investigated from the standpoint of cattle and poultry feeding and deserves further investigation from the standpoint of human nutrition.

The decrease in the B.V. and P.Q.I. on germination may be accounted for as follows:—In the general analysis of seeds it is seen that there is a loss in the total nitrogen due to germination. This will have its effect on the total digestible nitrogen. Further while estimating the protein quality index it was seen in this investigation (not published) that the water-soluble nitrogen and the undigestible nitrogen decrease on germination. As the water-soluble nitrogen has a lower nutritive value (Almquist, Stockstad and Halbrook, *loc. cit.*) than the digestible but water-insoluble nitrogen (*viz.*, nitrogen soluble in pepsin solution but insoluble in hot water) the biological value as well as the protein quality index will decrease on germination. Therefore the results obtained here fully justify the possible inferences (*viz.*, lowering of the P.Q.I. and B.V.). The increase in the digestibility coefficient due to germination can also be explained as the water-soluble nitrogen is all digested, though not completely retained by the animal body.

Considering the merits and the demerits of the foodstuffs investigated here, it is observed that the biological value as well as the protein quality index of cotton seed proteins compares well with those for other commonly used foodstuffs (*Health Bulletin* No. 23, Nutrition Research Laboratories, Coonoor, p. 49, 1941) and therefore they can supplement human protein requirements, if cotton seeds are made free from their gossypol content.

The proteins in lentil seem to be poorer as compared with the other two, cotton seed and Bengal gram. The protein quality index for this pulse is found to be varying between 50·81 and 57·18, where as this factor for cotton seed and Bengal gram lies between 59·23 and 70·89.

Summary

1. Comparison of the biological value obtained by animal experiments and the quality index of the protein obtained by chemical methods does show some parallelism between the two. How far it would be

applicable in general for the determination of the nutritive value of the proteins is difficult to see at the moment. This aspect has been investigated from the point of view of cattle and poultry feeding and deserves further investigation from the standpoint of human nutrition.

2. Germination appears to improve the digestibility coefficient of proteins in pulses, and the availability of vitamin C (unpublished work), though there is a lowering of the biological value. Hence the use of germinated pulses in food dishes.

3. Cotton seed contains good quality of protein and its nutritive value is also comparable with that of commonly used foodstuffs. It however contains a substance of poisonous nature (gossypol), and if it is removed cotton seed proteins can be used to supplement protein requirements of man.

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REFERENCES

Acharya, B. N., Niyogi, S. P. and Patwardhan, V. N. — *Ind. Jour. Med. Res.*, 1942, **30**, 73–80.

Almquist, H. J., Stockstad and Halbrook — *Jour. Nutr.*, 1935, **10**, 193.

Robert John Evans and St. John. J. L. — *Ibid.*, 1945, **60**, 331.

Chick, Hutchinson and Jackson — *Biochem. Jour.*, 1935 *a*, **29**, 1702.

Chick, Boas Fixson, Hutchinson and Jackson — *Ibid.*, 1935 *b*, **29**, 1712.

Martin and Robinson .. *Biochem. Jour.*, 1922, **16**, 407.

Mitchell .. *Ibid.*, 1924, **58**, 905.

Niyogi, Narayan and Desai .. *Ind. Jour. Med. Res.* 1931 *a*, **18**, 1217.

———— .. *Ibid.*, 1931 *b*, **19**, 475.

———— .. *Ibid.*, 1932, **19**, 1041.

Osborne and Mendel .. *Jour. Biol. Chem.* 1917, **32**, 369.

———— .. *Ibid.*, 1919, **37**, 559.

Swaminathan .. *Ind. Jour. Med. Res.*, 1937, **24**, 767.

AMIDE SYNTHESIS IN PLANTS

V. A Simple Method for the Preparation of Asparagine

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Received November 21, 1950

As an ingredient of media used for the cultivation of many micro-organisms asparagine finds frequent use in the laboratory and occasionally also in large-scale processes as in the production of tuberculin. On account of the comparative ease with which it crystallises out from solution even in the presence of large amounts of contaminating materials it is a fairly readily accessible product of plant metabolism. The method of its preparation has undergone little modification since its isolation by Piria and Schulze and other pioneer investigators of the role of this amide in protein regeneration in seedlings (for reviews on the early work on preparation of asparagine *see* Winterstein, 1923, Vickery and Schmidt, 1931 and Chibnall, 1939). In this method etiolated seedlings of legumes, such as *Lupinus albus* and *Lupinus angustifolius* which are usually used as its source, are macerated and extracted with water, the aqueous extract freed from protein either by heat coagulation or precipitation with lead acetate and the non-protein filtrate concentrated *in vacuo* till crystallisation begins. The asperagine thus obtained is freed from other amino-acids and amides formed during seed germination by careful fractional crystallisation. If the amount of impurities is large the asparagine along with any glutamine present is precipitated by mercuric nitrate and after decomposition of the mercury complexes by hydrogen sulphide the asparagine is separated from the more soluble glutamine by fractional crystallisation. Recently Vickery, Pucher and Deuber (1942) and Vickery and Pucher (1943) have described in detail a practicable procedure for the preparation of asparagine from *L. albus* and *L. angustifolius*. Dunn and Rockland (1947) also have indicated practical details found useful by them in this preparation. The methods used by these authors are essentially the same as that employed previously and the publications referred to are concerned mainly with defining with precision details of the various steps of the process such as germination of the seedlings, extraction, deproteinisation, filtration and crystallisation. Recently we had occasion to use the method of "alcoholic dialysis" described by Siddiqui (1945) for the preparation of biochanin C from the sprouts of germinated

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gram and found it to be a simple and elegant method for the preparation of asparagine. The experiments were initially taken up at the suggestion of Dr. S. Siddiqui to prepare biochanin C for the study of its growth promoting activity on animals. By alcoholic diffusion of 3–4 day old seedlings of Bengal gram (*Cicer arietinum*) a crystalline substance was isolated according to the method of Siddiqui, but that substance was found to have no growth-promoting activity on rats (Chanan Singh, 1950), nor did it agree with biochanin C in its elementary composition. The substance isolated by us was in fact found to be asparagine. In view of the ease with which the substance was obtained in pure crystalline condition further experiments were carried out on the possibility of using this method for the preparation of asparagine.

In applying alcoholic diffusion for this purpose it was obvious that conditions would have to be somewhat different from those originally used by Siddiqui and co-workers for the isolation of biochanin C, particularly with regard to the period of germination, which for the preparation of biochanin C was 4–5 days. It is well known that the accumulation of asparagine is not maximal at such an early stage of germination; in particular in the study of the amino-acid changes in germinating seedlings described in Part II of this series, it was shown by Damodaran, *et al.* (1946) that in *C. arietinum* asparagine and amide nitrogen increased up to the 16th day of germination. The alcoholic diffusion method was therefore applied to seedlings of different ages from 4–20 days. It was also necessary to exclude light during germination as it is known that asparagine accumulation is particularly marked when photosynthesis is absent. Experiments were also carried out to ascertain the completeness of the extraction.

In the sequel alcoholic diffusion was found to be a simple and convenient method for the preparation of asparagine. Compared to the usual aqueous extraction of macerated plant material, extraction of the intact seedling by diffusion with alcohol had the great advantage of not bringing into solution colloidal plant products such as proteins and complex carbohydrates, the removal of which is indispensible for crystallisation of asparagine. Under the conditions investigated extraction of diffusible constituents such as amino-acids and sugars was also found to be negligible. Crystallisation of asparagine from the alcoholic diffusate was extremely easy. Much of the material usually crystallised out of the extracts on standing in the cold room without previous evaporation or other treatment. From the point of view of yield also the method was very satisfactory, over 80% of the asparagine present in the seedlings, as determined by analysis, being obtained in pure

crystalline condition by three extractions. This represents on the weight of seed taken an yield of 5·0–5·5%. By continuing the extraction further yields could be improved, but this was not usually considered worthwhile. Yields of 10–20% have been previously recorded from *L. albus* and *L. angustifolius*. This is however of little importance as seeds of the lupin species mentioned are not readily available in quantity even in temperate climates (Dunn and Rockland, 1947) while Bengal gram is one of the commonest and cheapest of the pulses available in India. Experiments have nevertheless been planned to apply the alcoholic diffusion method to lupins and vetches available in India, as well as to stimulate amide accumulation in *Cicer arietinum* seedlings by physiological and chemical treatments.

It is interesting to note that a diffusion method for the preparation of asparagine using water as the solvent was described by Piutti (1925–26) although it does not appear to have come into general use.

Experimental

Germination of seeds.—A weighed quantity of Bengal gram was sterilised by immersion in 0·2% mercuric chloride solution for two minutes, washed several times to remove mercuric chloride, soaked in tap water for 24 hours and germinated in flats on acid-washed sand. The seed bed was kept moist by occasional spraying with tap water. In the initial experiments the germs from 4-day old seedlings were used for extraction and germination was carried out in diffused light. In later experiments the seedlings were etiolated by being grown in a dark room. After germination for the desired length of time the seedlings were removed from the sand, washed free from sand with water and adhering moisture removed by spreading the seedlings for a short period on sheets of filter-paper. The cotyledons were removed by hand-picking and the seedlings used for extraction as described below.

Extraction.—For extraction by diffusion the intact seedlings were placed in a flask, covered with methyl or ethyl alcohol and allowed to stand for 24 hours, at the end of which period the solvent was poured off and replaced with a fresh quantity. Three such extractions were usually found sufficient for practically complete extraction. To allow for loss of water from the seedlings during extraction with alcohol the concentration of alcohol used in successive extractions was 95, 80 and 70%.

Isolation.—A certain amount of crystalline material usually separated out from the first and second extracts on standing in the cold room. This was filtered off and the combined filtrates evaporated *in vacuo* at 40° C. till amorphous material started separating out. The solution at this stage was centrifuged and the supernatant liquid shaken up with a little ether to remove

ether-soluble materials which later interfere with crystallisation. The clear aqueous solution thus obtained was evaporated *in vacuo* to a thin syrup from which crystals separated on cooling. The crystalline material which was filtered off at the pump and washed in succession with cold water, alcohol, and ether consisted of practically pure asparagine from which the analytically pure substance was obtained by one recrystallisation.

Identification of Asparagine

(*a*) *Physical and chemical constants.*—The crystals as they separated when examined under the microscope consisted of large rhombs with hemihedral facets characteristic of asparagine. On slow heating the substance decomposed at 226° C.; on rapid heating in a sealed tube the melting point was found to be 234–35° C. The melting point was unaltered by admixture with an authentic sample of asparagine. Water of crystallisation determined by drying in vacuum over phosphorous pentoxide at 100° C. was 12·01% (theoretical for asparagine 12·0%). The elementary analysis (carried out by Dr. S. Subba Rao and Mr. G. R. Chaudhry of this Laboratory) gave the following values: C, 36·47%; H, 6·16%; N, 21·23%. Calculated for asparagine: C, 36·36%; H, 6·06%; N, 21·21%. Amino N by the Van Slyke method was: 5 minutes' shaking, 43·0% and 10 minutes' shaking, 49·45% of total N. Theoretical for asparagine: 50·00% of total N. Amide N determined according to the method of Chibnall and Westall (1932) as modified by Vickery, *et al.* (1935), was found to be 50·01%. Theoretical for asparagine 50·00%.

(*b*) *Isolation of products of acid hydrolysis.*—For further identification the substance was hydrolysed with dilute acid and after removal of ammonia, aspartic acid was isolated as the sole product of acid hydrolysis. 1·123 g. of the substance was hydrolysed by refluxing with 10 ml. of 3 N sulphuric acid for three hours. The hydrolysate was made alkaline with baryta and distilled *in vacuo* to about a third of the original volume to expel ammonia. After dilution it was freed quantitatively from Ba^{++} with sulphuric acid and evaporated *in vacuo* to crystallisation. The crystals which separated out were collected in several fractions and each crop after recrystallisation was identified as aspartic acid from the melting point and nitrogen content. M.P. 270–71° C.; N, 10·53% (for aspartic acid M.P. 270–71° C.; N, 10·52%).

(*c*) *Quantitative preparation of copper aspartate.*—0·4532 g. of the substance (dried to constant weight *in vacuo* over phosphorous pentoxide at 100° C.) was hydrolysed as before with dilute sulphuric acid and freed quantitatively from sulphuric acid and ammonia. The hydrolysate was made up to 150 ml. and 10 ml. withdrawn for determination of total N. The remain-

ing 140 ml. (containing 0·0897 g. of N) was heated to boiling with an excess of copper carbonate and filtered through a hot funnel. The residue was thoroughly washed with hot water and the filtrate and washings concentrated to small volume on a water-bath. After standing in ice for a short time the precipitated copper aspartate was filtered into a weighed crucible and the mother-liquor which was practically colourless was further evaporated to yield a second small quantity of copper aspartate which was added to the main precipitate. The combined copper salts after washing with cold water, alcohol and ether and drying in a vacuum desiccator weighed 0·7930 g., an yield of 99·59% on the basis of N content of 140 ml. of the solution.

Yield of asparagine from seedlings of various ages

In view of the fact that accumulation of asparagine in etiolated seedlings increases progressively for a considerable period of time and the demonstration by Damodaran, *et al.* (*loc. cit.*) that maximum asparagine production in Bengal gram is not reached before 16 days of germination preparation of asparagine was carried out from seedlings of various ages using the method of alcoholic diffusion as described above. Data relating to typical experiments are given in Table 1.

TABLE 1

Period of germination, days ..	4	7	14	18	20
Weight of dry Bemgal gram, g. ..	500	200	100	100	100
Weight of fresh seedlings, g. ..	280	580	680	700	710
Volume of solvent, l. ..	1·0	2·5	3·5	4·3	4·5
Weight of asparagine, g. ..	2·1	4·2	4·9	5·3	5·5
Yield of asparagine per 100 g. of dry seed, g. ..	0·42	2·1	4·9	5·3	5·5

It will be seen from the results recorded above that the highest yield was obtained at the end of 20 days' germination. It is possible that further prolongation of the experiment might have raised the yield of asparagine but by the 20th day the seedlings already showed signs of decay.

Completeness of extraction of asparagine by diffusion with alcohol

The completeness of extraction was tested on 18-day old seedlings in which asparagine N was determined on a small sample by the method described by Damodaran., *et al.* (*loc. cit.*). The bulk of the seedlings were then submitted to three extractions by diffusion with methyl alcohol. Asparagine N was again determined in a portion of the residual seedlings which

were then further extracted with methyl alcohol three times. The result given below refer to 100 g. of dry seed.

Weight of asparagine present in the seedlings as calculated from asparagine N .. .. .. 6·249 g.

Weight of asparagine isolated from fresh seedlings by extraction with methyl alcohol three times .. 5·3 g.

Weight of asparagine in the residual seedlings as calculated from asparagine N .. .. .. 0·52 g.

Weight of asparagine isolated from the residual seedlings by further extraction with methyl alcohol 0·48 g.

These results show that by three successive extractions by alcoholic diffusion the yield of asparagine is about 85%. The yield can be increased to about 92% by continuing the extraction three more times. In practice three extractions with methyl alcohol should be sufficient for the preparation of asparagine.

Summary

Details are given of a simple method for the preparation of asparagine by alcoholic diffusion of intact seedlings of Bengal gram (*Cicer arietinum*). The superiority of the diffusion method as compared with the usual aqueous extraction of macerated seedlings is indicated.

References

1. Chibnall and Westall .. *Biochem. J.*, 1932, **26**, 122.
2. Chibnall .. *Protein Metabolism in Plants* (New Haven), 1939.
3. Damodaran, Ramaswamy, Venkatesan, Mahadevan and Ramdas *Proc. Ind. Acad. Sci.*, 1946, **23**, 86.
4. Dunn and Rockland .. *Advances in Protein Chemistry*, 1947, **3**, 302–03.
5. ———— .. *Ibid.*, 1947, **3**, 359.
6. Piutti .. *Quoted from Abderhalden's Biochemisches Handlexikon, XII* (Berlin), 1930, 473.
7. Siddiqui .. *J. Sci. and Ind. Res.*, 1945, **4**, 68.
8. Singh .. *Thesis, the Punjab University*, Solan, 1950.
9. Vickery, Pucher and Clark .. *Biochem. J.*, 1935, **29**, 2710.
10. —— and Pucher .. *J. Biol. Chem.*, 1943, **150**, 197.
11. —— and Schmidt .. *Chem. Revs.*, 1931, **9**, 169.
12. ——, Pucher and Deuber .. *J. Biol. Chem.*, 1942, **145**, 45.
13. Winterstein .. *Handbuch der Biologischen Arbeitsmethoden*, 1923, Abt. 1, Teil, **7**, p. 73.

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A COMPARATIVE STUDY OF THE CONTRACTILE MECHANISM OF UNSTRIATED MUSCLE

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Singh and Singh (1949 *b*) have described the action of various substances on the contractile mechanism of unstriated muscle. They have further compared the effect of some substances on the contractile mechanisms of frog's and mammalian unstriated muscle; no significant difference between the two was found (Singh and Singh, 1950 *b*). In the present research the effect of ions on the contractile mechanism of *Mytilus* unstriated muscle on the one hand and of frog's and mammalian unstriated muscle on the other hand, have been compared, so as to elucidate whether the mechanisms of tonic contraction in the two kinds of muscle are identical or not. Reactions of isolated strips of myosin and unstriated muscle have been compared (Singh, 1943 *d*).

Methods

The muscles used were transverse strips from the stomach muscle of the frog *Rana tigrina*, and transverse pieces of dog's stomach muscle. The reactions of *Mytilus* muscle have been studied previously (Singh, 1938 *a*, 1939, 1943 *c*). The method of determining the action of substances on the contractile mechanism was as described previously (Singh and Singh, 1949 *b*, 1950 *b*), the reactions of dying muscles being determined.

Results

The environment of *Mytilus* muscle contains ions in much greater concentration than that of frog's or mammalian muscle. Ions have a very significant action on the contractile mechanism; it would, therefore, be of interest to know how the contractile mechanisms have adapted to the different concentrations of these ions found in the respective environmental media for *Mytilus*, mammalian and frog's muscles. The effect of high concentration of ions, as prevalent in sea water, has therefore been tested on frog's and mammalian muscle.

Effect of sodium salts

Effect of sodium chloride.—Sodium chloride, isotonic with frog's saline, 0·112 *M* NaCl, causes contraction of the contractile mechanism of frog's

muscle (Singh and Singh, 1949 *b*); 0·564 *M* NaCl, isotonic with *Mytilus* saline, causes elongation of the contractile mechanism of frog's muscle. Thus the length in 0·564 *M* NaCl of 6 frog's muscles was 123 ± 15 p.c. of that in frog's saline. Similarly 0·154 *M* NaCl, which is isotonic with mammalian saline, causes contraction of dog's stomach muscle (Singh and Singh, 1950 *b*); 0·564 *M* NaCl causes slight elongation of dog's stomach muscle, the length of 6 pieces being 104 ± 15 p.c. of that in mammalian saline.

In *Mytilus* muscle, 0·564 *M* NaCl causes contraction, which is more marked in winter muscles (Singh, 1938 *a*). Thus though the effect of high concentration of sodium chloride is not identical in the three kinds of muscle, it is more or less similar in their respective salines; the contraction of frog's and mammalian muscles, however, is not so marked as that of *Mytilus* muscle. Sodium chloride has, thus, more or less identical action on the contractile mechanisms of the three kinds of muscle. It is presumably responsible for tonus, as the sodium chloride content of isolated striated muscle, which shows no tone, is less than that of unstriated muscle which shows greater tone (Singh, 1938 *a*). Tone diminishes in sodium chloride deficient solutions (Singh, 1938 *b*).

In 0·564 *M* NaCl, winter *Mytilus* muscles contract, and then swell, but do not relax. Swelling therefore is not accompanied by relaxation. This phenomenon is much more marked in the next sodium salt to be considered, sodium cyanide. If calcium is present, then the *Mytilus* muscle relaxes in sodium chloride; calcium, therefore, antagonises the action of sodium chloride in all three kinds of muscle. Magnesium has similar action; so also small concentrations of potassium. It is interesting to note that G-actin, which is a globular protein, in the presence of certain concentrations of potassium and magnesium ions can be transformed into F-action, having fibrous form. Szent Györgyi's extracted fibre model is inhibited by calcium.

Effect of sodium cyanide.—Sodium cyanide, isotonic with frog's saline, 0·112 *M* NaCN, causes elongation of the contractile mechanism of frog's muscle (Singh and Singh, 1949 *b*); 0·564 *M* NaCN, isotonic with *Mytilus* saline causes elongation of the contractile mechanism of frog's muscle. Thus the length in 0·564 *M* NaCN of 6 frog's muscles was 152 ± 14 p.c. of that in frog's saline. Similarly 0·154 *M* NaCN causes elongation of dog's stomach muscle (Singh and Singh, 1950 *b*); 0·564 *M* NaCN causes marked elongation, the length of 6 pieces of dog's stomach muscle being 167 ± 21 p.c. of that in mammalian saline.

Sodium cyanide, 0·564 *M*, causes marked contraction of *Mytilus* muscle, though it is the most powerful substance in causing swelling. Thus elongation is not caused by swelling. The action of sodium cyanide is therefore quite different in *Mytilus* muscle on the one hand and frog's and mammalian muscles on the other.

Effect of sodium bromide.—Sodium bromide, 0·112 *M*, causes contraction of frog's muscle and 0·154 *M*, causes contraction of dog's muscle (Singh and Singh, 1949 *b*, 1950 *b*); 0·564 *M* NaBr causes no significant change in either muscle. Thus the lengths of 6 frog's muscles in 0·564 *M* NaBr were 80, 94, 114, 95, 100 and 113 p.c. of those in saline respectively, average being 99 p.c. The lengths of 6 dog's stomach muscles in 0·564 *M* NaBr were 100, 96, 100, 90, 93 and 104 p.c., average 97 p.c. In 0·564 *M* NaBr *Mytilus* muscle contracts strongly, but swells more than in sodium chloride. Thus swelling does not cause the muscle to relax.

Effect of sodium nitrate.—Sodium nitrate, 0·112 *M*, causes contraction of frog's muscle, and 0·154 *M* causes contraction of dog's muscle (Singh and Singh, 1949 *b*, 1950 *b*); 0·564 *M* $NaNO_3$ causes elongation of frog's muscle, and no significant change in dog's muscle. Thus the length of 6 frog's muscles in 0·564 *M* was 132 $\pm$ 19, and that of 6 dog's muscles, 97 $\pm$ 21. In 0·564 *M* $NaNO_3$, as in chloride and bromide, *Mytilus* muscle contracts strongly, but swells more than in bromide. Thus swelling does not cause the muscle to elongate.

Effect of sodium iodide and thiocyanate.—Sodium iodide 0·112 *M*, and 0·154 *M* has no significant effect on frog's and dog's muscles respectively. In 0·564 *M*, both muscles elongate, their length being 147 $\pm$ 15 and 112 $\pm$ 11 p.c. of that in saline respectively. In 0·564 *M* NaI, *Mytilus* muscle swells as well as relaxes, the action being identical in all the three kinds of muscle.

Sodium thiocyanate causes swelling as well as elongation of the three kinds of muscle.

Effect of potassium salts

Solutions of potassium chloride, isotonic with their respective salines, cause swelling and relaxation of all the three kinds of muscle. In 0·564 *M* KCl, frog's muscle does not show any significant change, though the dog's muscle contracts. Thus the length of 6 frog's muscles in 0·564 *M* KCl was 103 $\pm$ 10 p.c. of that in saline, and of 6 dog's muscles, 86 $\pm$ 8 p.c. This shows that for comparison of the effects on the three kinds of muscle, solutions which are isotonic with their respective salines, have to be taken into consideration.

Potassium overcomes the effect of anions. Thus *Mytilus* muscle relaxes in KBr, KNO_3 and KCN. Sodium salts, therefore, cause fixation of the contractile mechanism at certain length and potassium undoes that fixation. Thus in the sodium salts, the muscle swells in the fixed condition, whilst in the presence of potassium, the fixation is undone, so that the swelling is able to relax the muscle.

The above view is derived from the following experiment. If an unloaded frog's rectus abdominis is immersed in isotonic potassium chloride solution, it contracts and then gradually spontaneously returns to its original length in the presence of the potassium chloride solution. As relaxation in striated muscle has been shown to be passive (Hill, 1949), the spontaneous return of the muscle to its original length must be ascribed to swelling, otherwise relaxation in striated muscle would have to be considered as active. Relaxation of striated muscle fibres in acid solutions has been ascribed to swelling (Barer, 1947).

Solutions of ammonium chloride isotonic with their respective saline, cause relaxation of *Mytilus* and frog's muscles and contraction of dog's muscle. In 0·564 *M* NH_4Cl, frog's muscle relaxes and dog's muscle contracts.

Effect of divalent ions

Effect of barium chloride.—Barium chloride (0·376 *M*) causes a very powerful contraction of the contractile mechanism of *Mytilus* muscle. The muscle at first loses weight but thereafter gains weight (Singh, 1938 *a*); it never relaxes. In contrast to the action on *Mytilus* muscle, barium chloride solutions isotonic with their respective salines, cause elongation of frog's and dog's muscle (Singh and Singh, 1949 *b*, 1950 *b*). Thus the action of barium is quite different on *Mytilus* muscle on the one hand, and frog's and dog's muscle on the other hand. In *Mytilus* muscle, barium chloride produces a contraction which lasts till the death of the muscle (Singh, 1938 *d*).

Barium chloride, 0·376 *M*, has no significant action on frog's muscle, but produces contraction of the contractile mechanism of dog's muscle. Thus the lengths of 6 frog's muscles was 101 $\pm$ 8 p.c. of that in saline, and of 6 dog's muscles, 76 $\pm$ 5 p.c.

Effect of calcium chloride.—Calcium chloride causes contraction of the contractile mechanisms of all the three kinds of muscle; 0·075 *M* causes contraction of frog's muscle, 0·103 *M*, that of dog's muscle and 0·376 *M*, that of frog's, dog's and *Mytilus* muscles.

Effect of strontium chloride.—Strontium chloride, 0·075 *M*, causes contraction of frog's muscle, 0·103 *M* causes contraction of dog's muscle, but 0·376 *M* causes relaxation of the contractile mechanisms of frog's and *Mytilus* muscles and contraction of dog's muscle.

Effect of magnesium chloride.—Magnesium chloride solutions, isotonic with their respective salines, cause relaxation of the contractile mechanism of all the three kinds of muscle.

Dissociation of the excitatory and contractile mechanisms

The excitatory and the contractile mechanisms can be dissociated by methods other than that described previously (Singh and Singh, 1949 *b*).

Effect of high voltages.—Both frog's and dog's muscles can be stimulated with high voltages of alternating current without any apparent harm. Thus they were stimulated with 110, 220, 440 and 880 volts. If the frog's muscle was stimulated with 110 volts, it became inexcitable to ordinary stimuli (10 volts). If stimulated with 220 volts, then it became inexcitable to 110 volts; if stimulated with 440 volts it became inexcitable to 220 volts and so on.

The contractile mechanism does not appear to be affected, as the contraction appears to be normal in every way (Fig. 1). Relaxation may

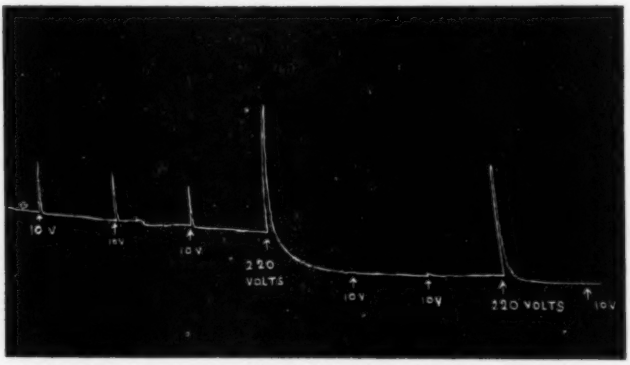


FIG. 1. Frog's stomach muscle. Stimulated with 10 volts, alternating current for 10 seconds and 220 volts.

decrease or increase. It must be the excitatory mechanism therefore, which is affected. There is a marked difference between the various muscles in their behaviour towards high voltages. Some muscles become inexcitable to ordinary stimuli after 110 volts, others require 220 volts or still stronger stimuli. In a muscle rendered inexcitable, tone also decreases.

A frog's muscle can be rendered inexcitable by passing 220 volts for 15 minutes, the temperature of the muscle chamber being not allowed to rise above 35° C. by flow of saline. After such treatment, the muscle slightly contracts. If, however the temperature is allowed to rise, unloaded muscle, actively elongates just before death.

In frog's muscle, which had been rendered inexcitable by the above method, chlorides of sodium, potassium, barium, calcium and urea produced the usual effect on the contractile mechanism, so that these experiments support the view expressed previously that in dying muscles, it is the contractile mechanism which is affected by various substances.

Effect of methyl alcohol.—Methyl alcohol also destroys excitability, but not the contractile mechanism, as the muscle contracts when stimulated with high voltage (220 volts). The concentration of methyl alcohol required to make a frog's muscle completely inexcitable and diminish tone is variable; some muscles require 1 c.c. per 100 c.c. of saline, whilst others require about 15 c.c. added to 85 c.c. of saline. So in these experiments 20 c.c. added to 80 c.c. of saline was used to destroy excitability.

The action of the above saline is to cause the muscle to contract; this contraction must be due to the action on the contractile mechanism. The action of following substance was tested on the contractile mechanism of frog's muscle—potassium chloride, calcium chloride, sodium chloride and urea. Potassium chloride produced a slight relaxation, sodium chloride a slight contraction, calcium chloride and urea having no significant action. The method however is not suitable, owing to the strong contractile effect of methyl alcohol. So substances do not show a contractile effect, unless their action is stronger than that of methyl alcohol, and they also do not produce an elongating effect, unless their action is greater than the antagonising effect of methyl alcohol. The above experiments show that the effect of potassium and sodium is on the contractile mechanism.

Cold, 0–3° C. may render some frog's muscles inexcitable, but the action is not powerful.

The relation between tone and asphyxial contraction

It was stated that the asphyxial contraction was identical with one of the tonic contractions, exhibited normally by unstriated muscle (Singh, 1949; Singh and Singh, 1947, 1948 *a*, *b*; 1949 *a*, *c*). This has been proved by another experiment. If a piece of dog's muscle is put under tension of about 50–60 g., and this tension destroyed by forcible stretching, the process

being repeated twice or thrice, so that a total of 100–150 g. tension is destroyed, then the twitch contraction by alternating current (Singh and Singh, 1950 *a*) and potassium is unaffected but the asphyxial contraction never occurs (Fig. 2). In these experiments, the temperature of the muscle must not be allowed to fall below 20° C., as may happen during dissection, handling or otherwise. Preferably, the room tempeature should be 37–38° C.

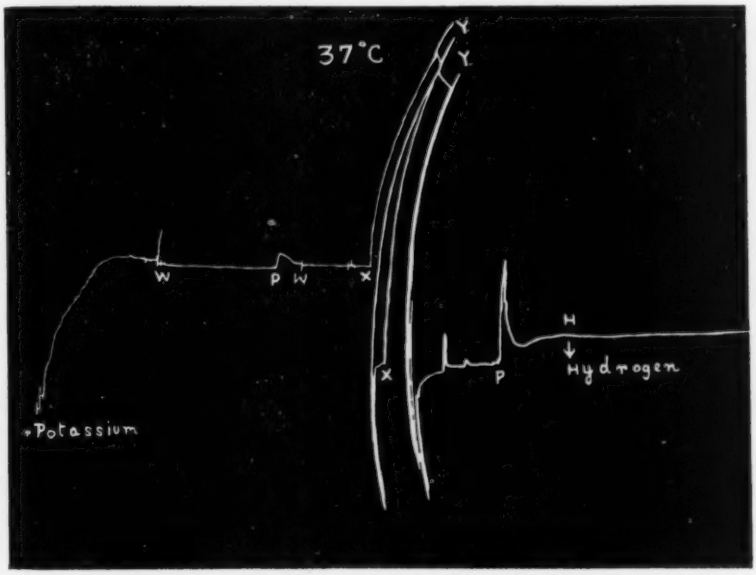


FIG. 2. Dog's stomach muscle. 37° C. stimulated with potassium, 0·06 *M*, at P. Potassium withdrawn at W. Muscle put under tension at X, and tension forcibly destroyed at Y. Muscle asphyxiated at H.

If the temperature of the muscle is allowed to fall, then the contractile mechanism may be permanently damaged and the asphyxial contraction does not occur, even if the temperature of the muscle is subsequently restored to 37° C.

It will be noticed that prior to stretching, relaxation of the contraction produced by potassium is very slow, but after stretching, potassium is unstable to produce a sustained contraction, though the twitch is unaffected. This shows that the twitch and the sustained contraction are produced by different contractile mechanisms. This is further shown by repeating and the experiment at 30° C. at which temperature the asphyxial contraction in dog's muscle is slight and is absent at 20° C. (Singh and Singh, 1949 *c*). It will

be noticed that potassium is unable to produce sustained tension, and afte stretching the twitch increases (Fig. 3). Any sustained tension is due to lactic tone, as it diminishes on asphyxiation or withdrawal of potassium. If the muscle is jerked during twitch contraction, then the twitch mechanism

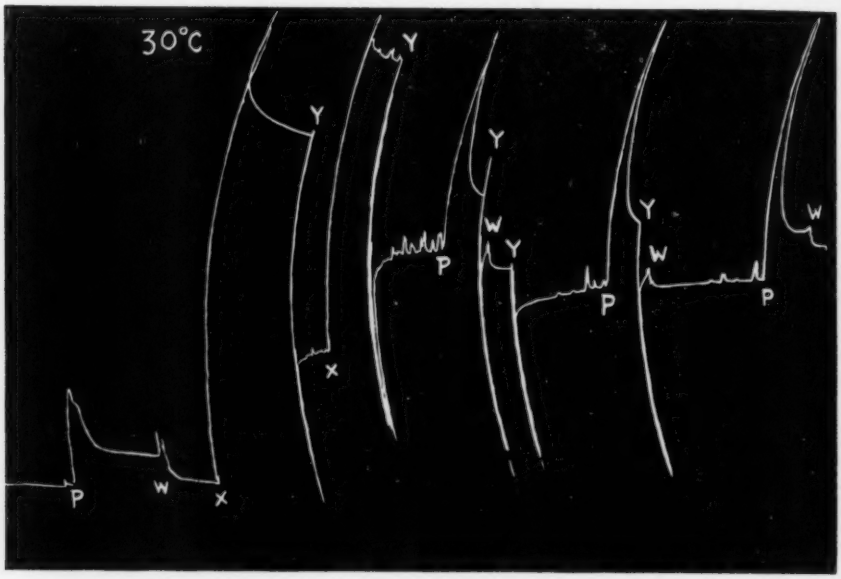


FIG. 3. Dog's stomach muscle. 30° C. stimulated with potassium at P. Potassium withdrawn at W. Put under tension at X and tension destroyed at Y.

is damaged. The above inactivation at 20° C. occurs when the muscle is stimulated with potassium or other stimulants. Otherwise cold produces a tonic contraction of the muscle due to direct effect on the contractile mechanism, as shown by the fact that the contraction is resistant to cyanide or asphyxia. If the contraction is due to the excitatory mechanism, then it is diminished by asphyxia. Cold may produce both these effects (Singh and Singh, 1949 *a*).

DISCUSSION

The basic fact that emerges from these experiments is that the contractions of the contractile mechanism of unstriated muscle can be divided into two groups, those which can be undone by swelling and those which cannot be so affected. In the first instance, the muscle swells and relaxes actively, and in the second instance, it swells in a contracted state; by the words "relaxes actively", here is implied that no external force is used to cause

relaxation, though the muscle may be relaxed by the internal force of swelling.

How are these facts to be explained? It is clear that in these two instances, the contractile proteins in the muscle are in different internal configurations. The protein actin, first isolated by straub, can exist in two different forms. One of these, the form in which the protein is extracted, is that of globular protein (" G-actin "), which in the presence of certain concentrations of potassium and magnesium ions can be transformed into " F-actin " having a fibrous form. Both G-actin and F-actin can combine with myosin, forming respectively G-actomyosin and F-actomyosin.

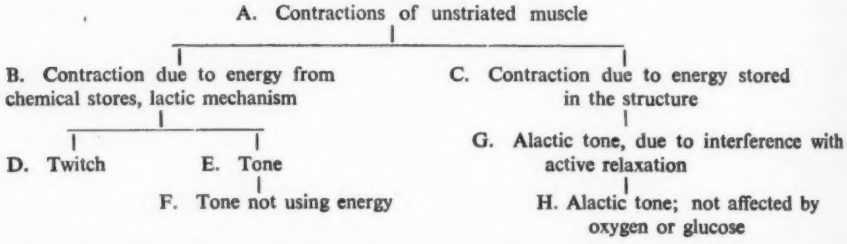
There is a close parallelism between the phenomena exhibited by the muscle and by the isolated protein; so it may be stated that the proteins of muscle during contraction exist in two forms, one fibrous and the other globular, as in one case the muscle swells longitudinally and in the other case, " globularly ". The " globular " state can maintain a state of tension or persistent contraction without expenditure of energy, and thus constitutes the " catch mechanism " of unstriated muscle. In the muscle, potassium and magnesium release the catch mechanism and in solution, they convert G-actomyosin into F-actomyosin. In *Mytilus* muscle, substances which produce the " globular " swelling, also produce a contraction which is not susceptible to asphyxia, and potassium neutralises this contraction (Singh, 1943 *b*, Figs. 2, 3). Normal tone in *Mytilus* muscle also consists of a similar state, as potassium has identical action (Singh, 1938 *c*, Fig. 6; 1943 *a*, Fig. 7). Calcium also antagonises the globular state; alternating current has also a similar action, presumably acting by liberation of calcium (Singh, 1939, 1944). Szent-Györgyi's extracted fibre model is inhibited by calcium.

It is now realised that folding and unfolding of linear protein molecules are responsible for the contraction and relaxation of muscle, and that the spherical molecule is a much folded chain. The catch mechanism can be undone by stretching and it is interesting to note that alpha keratin changes to beta keratin on stretch. The proteins of wool and hair are straight when wet and curl when dry. This parallels the behaviour of unstriated muscle described above, as it straightens out when hydrated and contracts when dehydrated.

According to Szent Györgyi, the contraction of actomyosin in the presence of ATP is a process of dehydration. This view is supported by experiments on unstriated muscle, which relaxes on hydration and contracts

on dehydration. It is interesting to note that in *Mytilus* muscle, small concentrations of potassium (0·1 *M* KCl) produce contraction, whilst larger concentrations (0·4–0·5 *M*) produce relaxation (Singh, 1938 *c*) Szent Györgyi observed that small concentrations of potassium cause contraction of actomyosin, and larger concentrations, relaxation. Szent Györgyi observed that the actomyosin system can also be activated by magnesium. In living unstriated muscle, small concentrations of magnesium increase the excitatory effect of other substances, and in dog's muscle, magnesium chloride produces contraction (Singh, 1949 *a*). Varga (1946) has observed that actomyosin threads shorten maximally at 16–18° C. It is interesting to note that tone in unstriated muscle shows a maximum at about 15–20° C., the contraction produced in *Mytilus* muscle by potassium, at about 15° C., and that by alternating current at about 20° C. In frog's muscle, the optimum temperature for contraction produced by alternating current is about 20–30° C. (Rao and Singh, 1940).

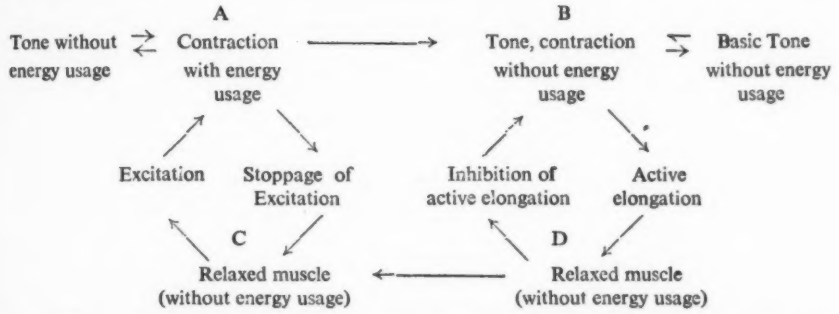
The special mechanism of tonus described above is probably not found in mammalian and frog's muscle, as "globular" swelling has not been noticed in them. It is a special mechanism developed in *Mytilus* muscle and probably in other molluscs. The relation of this mechanism, however, to tonus produced by interference with active relaxation has yet to be elucidated; they may or may not be identical. The contractions of unstriated muscle can be classified as follows:—


A. Contractions of unstriated muscle
B. Contraction due to energy from chemical stores, lactic mechanism
C. Contraction due to energy stored in the structure
D. Twitch
E. Tone
F. Tone not using energy
G. Alactic tone, due to interference with active relaxation
H. Alactic tone; not affected by oxygen or glucose

The G contraction represents the normal tone of unstriated muscle, being due to interference with active relaxation. It is the tone which increases when the temperature is lowered. The H contraction is the one, the formation of which is prevented by lowering the temperature to 20° C., though once formed, it is not affected by such a procedure (Singh and Singh, 1949 *c*).

The difference between the G and H contractions is that the latter is not amenable to inhibitory influences. It yields to stretch. It is the basic tone found in all muscles, because inhibitory agencies never cause complete relaxation of unstriated muscle; there is always some residual tone left

which may form a small or great part of the total tonus. Its magnitude can be judged by applying an inhibitory stimulus. Thus in muscles, which are known ordinarily to be sensitive, adrenaline may be used. The latter causes great relaxation in some muscles and very little in others. Similarly, electric current may be used. This basic tone may be responsible for spastic states in the human being, such as vascular hypertension, spastic colon, etc. Electric current may also be used in some muscles to cause inhibition. The F contraction readily yields to inhibition. The F and H contractions are not identical as the H contraction is relaxed by sodium cyanide or barium chloride. Our previous scheme (Singh and Singh, 1949 *a*) is slightly modified as follows:—



The natural tendency for unstriated muscle is to shorten. As unstriated muscle is a primitive tissue, it would appear that the system BD is a primitive one, which has been modified to the system AC. In unstriated muscle both these systems are present, but in striated muscle only system AC is present, as there is no active relaxation. But as the muscle dies, the system AC passes into the system BD, thus causing rigor mortis in striated muscle, just as the primitive gasping centre becomes active in respiratory failure.

It is interesting to note that substances may produce quite opposite effects by acting on the contractile mechanism directly and indirectly through the excitatory mechanism. Thus barium chloride is a powerful stimulant, but relaxes the contractile mechanism; similarly, potassium, thiocyanate, etc., produce different effects. The action of adrenaline and acetylcholine are identical whether acting directly or indirectly on the contractile mechanism (Singh and Singh, 1950 *b*, *c*).

An interesting fact that comes to light is that the proteins which have been compared above with unstriated muscle have been extracted mostly from striated muscle. Their similarity in behaviour to unstriated muscle shows, that the constitution of the contractle mechanism in the latter is

more primitive than in the former, as during chemical extraction, complexities of structure are likely to be broken and not reconstituted in artificial models.

In a recent letter, Katchalsky and Eisenberg (1950) refer to a model of muscle consisting of a polyvinyl phosphate thread which contracts strongly in concentrated acid and expands rapidly to its original length when returned to distilled water and alkali. Unstriated muscle also contracts in strong acids and relaxes in distilled water or dilute alkali by action on the contractile mechanism (Singh and Singh, 1950 *b*).

The question arises as to how our previous findings regarding " viscosity " fit into the new scheme. if a substance unlocks the contractile mechanism, such as for example potassium, then the rate of extension will be increased, thus simulating decrease of viscosity. So substances which we have mentioned previously to decrease the viscosity, have thus an unlocking action on lactic or alactic tone. Muscle viscosity may however be concerned in a nonspecific way, such as when temperature is lowered, the movements are slowed. But this may be due to slower turnover of energy, which will diminish the rate of contraction as well as relaxation, as for example happens in striated muscle during fatigue.

Summary

1. A comparative study of the contractile mechanism of frog's, dog's and *Mytilus* muscles has been made.

1. Sodium and potassium chlorides have identical action on the three kinds of muscles. Barium chloride and sodium cyanide cause contraction of the contractile mechanism of unstriated muscle of *Mytilus* and elongation of frog's and dog's muscle.

3. Contraction of the contractile mechanism of unstriated muscle are of two kinds. One kind is relaxed by swelling of the muscle and the other kind not. Relation of this finding to the tonus mechanism is discussed.

4. Action of ammonium, calcium, strontium, magnesium, bromide, nitrate, iodide and thiocyanate is described. Potassium, magnesium relax the contractile mechanism.

5. The excitatory and the contractile mechanism of unstriated muscle have been dissociated by destroying the former by high voltages (110–880 volts A.C.) and by methyl alcohol. Action of substances on the contractile mechanism has then been studied.

6. If normal tone of the muscle is destroyed, then the asphyxial contraction does not occur, proving the identity of the two.

References

Barer, R. .. .. .. *J. Anat.*, 1947, **81**, 259.

Rao, M. S. and Singh, I. .. *J. Physiol.*, 1940, **98** , 12.

Katchalsky, A. and Eisenberg, H. *Nature*, 1950, **166**, 267.

Hill, A. V. .. .. .. *Proc. Roy. Soc.*, 1949, **136B**, 399.

Singh, I. .. .. .. *J. Physiol*, 1938 *a*, **91**, 398.

—— .. .. .. *Ibid.*, 1938 *b*, **92**, 62.

—— .. .. .. *Ibid.*, 1938 *c*, **92**, 232.

—— .. .. .. *Ibid.*, 1938 *d*, **92**, 241.

—— .. .. .. *Ibid.*, 1939, **96**, 1.

—— .. .. .. *Proc. Ind. Acad. Sci.*, 1943 *a*, **17**, 20.

—— .. .. .. *Ibid.*, 1943 *b*, **17**, 143.

—— .. .. .. *Ibid.*, 1943 *c*, **28**, 53.

—— .. .. .. *Nature*, 1943 *d*, **152**, 192.

—— .. .. .. *Proc. Ind. Acad. Sci.*, 1944, **29**, 91.

Singh, S. I. and Singh, I. .. *Curr. Sci.*, 1947, **16**, 259.

———— .. .. *Proc. Ind. Acad. Sci.*, 1948 *a*, **27**, 127.

———— .. .. *Curr. Sci.*, 1948 *b*, **17**, 321.

———— .. .. *Proc. Ind. Acad. Sci.*, 1949 *a*, **30**, 343.

———— .. .. *Ibid.*, 1949 *b*, **30**, 270.

———— .. .. *Ibid.*, 1949 *c*, **30**, 263.

———— .. .. *Ibid.*, 1950 *a*, **31**, 351.

———— .. .. *Ibid.*, 1950 *b*, **32**, 12.

———— .. .. *Curr. Sci.*, 1950 *c*, **19**, 60.

Vorga, L. .. .. .. *Hung. Acta. Physiol.*, 1946, **1**, 1. Quoted from Sandow, A., *Ann. Rev. Physiol.*, 1949, **11**, 297.

CATION EXCHANGE CAPACITY OF *AVARAM* RESIN

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(Communicated by Prof. M. S. Thacker, F.A.Sc.)

In order to study the property of the *Avaram* Cation exchanger prepared in this laboratory from the bark of *Cassia auriculata* (Venkataramanan, 1945) a series of comparative experiments were conducted with standard materials. *Amberlite I.R.* 100 (a cation exchanger kindly supplied by the Resinous Products Co., Philadelphia) and *Zeokarb* (the carbonaceous exchanger supplied through the courtesy of Permutit Co., London) were used for the comparative studies.

Experimental

The capacities of the individual resins for exchanging calcium ions were determined on the hydrogen and sodium cycles of operation from a solution of calcium chloride. Both the percolation and equilibrium studies were conducted.

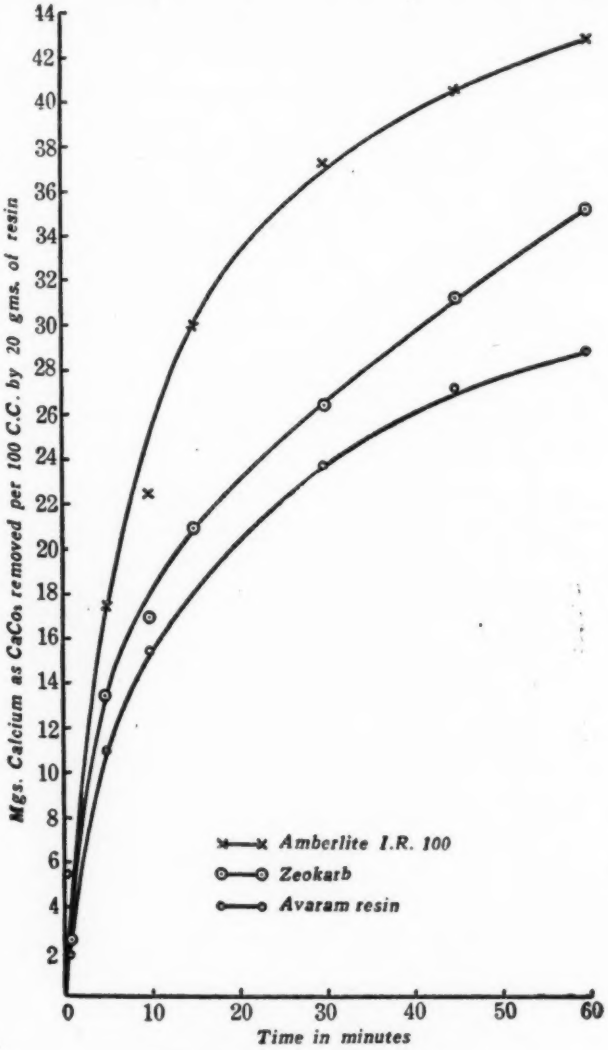
Percolation method.—20 grams. of the resin (+ 20, − 40-mesh size) were packed in a narrow cylindrical glass tube (diameter 2·22 cm.). The resin was regenerated either with 10% hydrochloric acid (for the hydrogen cycle) or 5% sodium chloride solution (for the sodium cycle) at a rate of 23·7 gallons per square foot per hour (7·3 c.c./minute). When the resin was washed free of the regenerant, calcium chloride solution was percolated at the same rate. The exchange capacity was determined after passage of 2,500 c.c. of the solution in each case.

The effluents were collected in lots of 250 c.c. and they were analysed for the calcium content volumetrically against standard permanganate. The titratable acidity produced on the hydrogen cycle of operations was determined for each lot collected.

Equilibrium Studies.—The resin was regenerated as above and later allowed to dry overnight at room temperature. 20 gm. of the resin were placed in a large glass bottle and 2,000 c.c. of calcium chloride solution was added. 50 c.c. lots of the resulting solution were pipetted out into dry conical flasks at intervals of 1, 2, 3, 4, 5, 10, 15, 30, 45 and 60 minutes from the start. The contents were kept agitated and the temperature varied

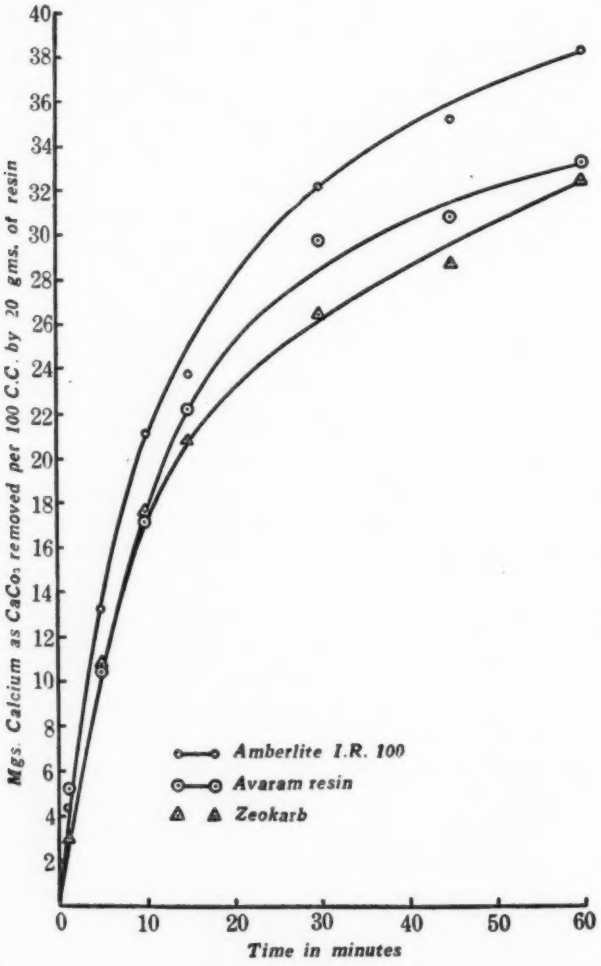
between 25 and 27° C. The solutions were analysed for their calcium content and the titratable acidity was noted on the hydrogen cycle of operations.

The above technique was also adopted to study the influence of contact with a solution at 60° C. on the capacities of *Avaram* and *Amberlite I.R.* 100



Experiments on Hydrogen Cycle
GRAPH I. Temp. 25–27° C.

when working on the hydrogen cycle. The modified procedure was as follows:—Calcium chloride solution was warmed to nearly 60° C. and then poured into the reaction vessel containing the resin. The system was maintained at 58° C. with constant agitation and the rest of the procedure was as before.



Experiments on Sodium Cycle
GRAPH II. Temp. 25–27° C.

Results

Table I gives the capacities of the resins on the hydrogen and sodium cycles of operation obtained by the percolation technique.

The capacity of the resin denotes the milligrammes calcium as calcium carbonate which has been removed by one gram of the resin.

Table I

Material	Capacity on	
	Hydrogen cycle	Sodium cycle
Amberlite I. R. 100 ..	51·5	46·3
Zeokarb ..	48·0	45·2
Avaram resin ..	35·0	40·0

Graphs I and II represent the progress of calcium removal in the equilibrium studies. The removal in the hydrogen cycle is represented by Graph I while Graph II represents removal on the sodium cycle.

Table II (*a*) gives the results of the experiments on the influence of contact with a hot solution on the capacity of *Avaram* and *Amberlite I.R.* 100 working on the hydrogen cycle. To study if the resins had been deleteriously affected, the used resins were regenerated and again worked at normal temperature and the results obtained are given in Table II (*b*).

Table II

(*a*)

Material	Temperature °C.	Milligrammes calcium removed as $CaCO_3$ by 20 gm. of resin in 100 c.c. solution in time (minutes)						
		1	5	10	15	30	45	60
Amberlite I. R. 100	26	5·5	17·5	22·6	30·0	37·5	40·7	43·1
	58	6·9	18·9	28·8	35·0	41·4	42·6	44·0
Avaram resin ..	26	2·0	11·1	15·6	20·7	23·8	28·0	29·0
	58	5·6	12·0	20·2	25·7	28·5	29·5	29·5
(*b*)		After regeneration						
Amberlite I. R. 100	26	5·6	16·8	22·0	31·0	37·0	42·0	44·6
Avaram resin ..	26	2·2	12·0	15·0	21·0	24·0	28·8	29·8

Results from Table III shows that the acidity produced in the hydrogen cycle of operation (Equilibrium experiments) is proportional to the amount of calcium found to be removed. A few readings are given to show the relationship.

TABLE III

Material	Time in minutes	Acidity as $CaCO_3$ in 100 cc. solution	Calcium as mg. $CaCO_3$ removed from 100 c.c. solution
Amberlite I.R. 100	1	4·8	5·5
	10	23·0	22·6
	30	36·8	37·5
	60	43·2	43·1
Zeokarb ..	1	3·0	2·5
	10	16·0	16·9
	30	27·0	26·5
	60	36·0	35·4
Avaram resin ..	1	3·0	2·0
	10	15·5	15·6
	30	24·0	23·8
	60	29·6	29·0

DISCUSSION AND SUMMARY

Hydrogen Cycle.—Percolation and equilibrium studies reveal that the capacity of the *Avaram* resin is lower than that of *Amberlite I.R.* 100 and *Zeokarb*.

The acidity developed during exchange reaction has been found to be proportional to the amount of calcium removed and serves as an index for studying the course of calcium removal. This confirms the findings of Myers and Eastes (1941) who stated that " the quantitative relation which exists between the cations adsorbed and the liberated acid " could be utilised to determine the total cations in a mixture or a single cation from a pure solution as in this case.

Sodium Cycle.—The capacities of the three resins in the percolation and equilibrium experiments follow the same order as found in the hydrogen cycle. While the capacities of *Amberlite I.R.* 100 and *Zeokarb* are slightly lower on the sodium cycle, the capacity of the *Avaram* resin alone shows an increase over the hydrogen cycle (Table I).

Nachod and Wood (1944) reported that the capacities of *Amberlite I.R.* 100 and *Zeokarb* were lowered on the sodium cycle. It is not possible to explain the slightly increased capacity of the *Avaram* resin when operating on the sodium cycle. Incidentally this comparative study reveals that

Amberlite I.R. 100 has a higher capacity than *Zeokarb* in the sodium cycle contrary to the claim of Nachod and Wood (*loc. cit.*).

Stability of Avaram *Resin.*—Results from Table II (*a*) indicate that there is no appreciable increase in the overall exchange by the *Avaram* and *Amberlite* Resins but only a slight increase in the initial stages of the reaction. This agrees with the findings of Nachod and Wood (*loc. cit.*) who stated that a temperature range between 27° C. and 60° C. does not alter the equilibrium constant. That the treatment at 58° C. has left no deleterious effects on the exchange capacity of the resins is revealed by results in Table II (*b*).

Thus, the *Avaram* resin is found to behave like a cation exchanger possessing stability, yielding acid quantitatively in the calcium-hydrogen exchange cycle but possessing a slightly higher capacity on the sodium cycle of operation.

ACKNOWLEDGMENT

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REFERENCES

1. R. J. Myers and J. W. Eastes .. *Ind. Eng. Chem.*, 1941, **33**, 1203.
2. F. C. Nachod and W. Wood .. *J. Am. Chem. Soc.*, 1944, **66**, 1380.
3. K. Venkataramanan .. 1945—Unpublished data.

ACTIVE RELAXATION OF UNSTRIATED MUSCLE PRODUCED BY ADRENALINE

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Active relaxation of muscle has recently aroused considerable interest owing to its bearing upon the theories of muscular contraction. Kuhne (1859) found that a frog's sartorius laid on a mercury surface and stimulated did not return to its original length unless pulled out. Kaiser (1900) found the opposite effect. Ramsey and Street (1940, 1941) working on isolated single muscle fibres reported that relaxation was active. Hill (1949) conclusively proved by a very sensitive method, that relaxation in striated muscle was passive. Active relaxation, however, has been discovered in unstriated muscle (Singh and Singh, 1948, 1949*a*, 1950 *a*, *b*, 1951). Frog's stomach muscle relaxes actively when treated with adrenaline (Singh and Singh, 1950 *a*); guinea pig's uterus also behaves similarly (Singh and Singh, 1951).

In the present research, active relaxation of frog's stomach muscle by adrenaline, hydrogen ions and increase of osmotic pressure of the saline is described. The movements of unstriated muscle, compared to those of striated muscle, are very sluggish, so that complicated apparatus is not necessary to follow contraction and relaxation. These experiments with adrenaline are most satisfactory in demonstrating active relaxation of muscle, as there is no preliminery contraction, except that of normal tone, and the muscle just lengthens.

Methods

Transverse and longitudinal pieces from the stomach muscle of the frog *Rana tigrina* were used (Singh, 1939). The composition of the saline was as described previously (Singh, 1939), buffered with borate, the pH being 8·1. The muscle was placed in a petri dish containing saline to which adernaline was added. The length of the muscle was measured before and after 20 minutes of adding adrenaline chloride, the muscle being absolutely untouched throughout the experiment. If the muscle curled, then it was kept pressed by keeping a glass rod on its central portion, so that it was free to expand at both ends. The experiments were performed at 25–27·5° C., this being the optimum temperature for active relaxation.

Rabbit's gut was also used; it was slit longitudinally and the action of adrenaline tested.

Results

On addition of adrenaline, the circular muscle of frog's stomach begins to lengthen almost immediately; the lengthening is completed in 10 minutes, so that the time of observation in these experiments was fixed at 20 minutes. The muscle actively relaxed in all concentrations of adrenaline tested, the experimental range being 1 in 10 million to 1 in 40,000. Maximum elongation was achieved in concentration of 1 in million; therefore the concentration chosen for experiments in this paper was 1 in 40,000 to 1 in 100,000. Thus at 23·5° C., in increase of length of 6 muscles was 12 ± 5 p.c. with adrenaline 1 in 10 million, 15 ± 6 p.c. with 1 in million, 15 ± 3 p.c. with 1 in 100,000 and 14 ± 3 p.c. with 1 in 40,000.

Compared to circular muscle fibres, longitudinal fibres lengthen very little. Thus in 20 experiments, 10 showed no change in length, whilst the rest lengthened by 4 to 9 p.c. The reaction of longitudinal fibres was tested to exclude lengthening of circular fibres by contraction of these fibres, if at all there was any such effect. The longitudinal fibres near the cardio-œsophageal sphincter relax somewhat. Thus in 8 experiments, 2 muscles showed no change, whilst 6 increased in length by 5 to 25 p.c. The experiments in this paper were, therefore, performed on circular muscle.

The effect of adrenaline was tested on different portions of the frog's stomach. The latter was divided transversely into 5 equal parts; the first part included a bit of the œsophagus and the cardio-œsophageal sphincter. The last 2–3 mm. of pylorus was rejected. The results are shown in Table I. It will be seen, that in many stomachs, the pylorus does not relax. This is due to the fact that this part of stomach frequently shows alactic tone, which is not sensitive to adrenaline, so that it neither relaxes actively nor passively (Singh and Singh, 1950 *c*). Passive relaxation by adrenaline occurs most readily in the cardio-œsophageal part, but this is not the most sensitive part if tested for active relaxation.

Rabbit's gut, though it relaxes passively when treated with adrenaline, does not relax actively at all (6 experiments). Therefore tonus of unstriated muscle can be divided into 3 kinds, when active or passive relaxation is produced by adrenaline: (1) The muscle does not relax actively or passively. (2) The muscle relaxes actively as well as passively. (3) The muscle relaxes passively but not actively.

Effect of cyanide.—Sodium cyanide (1 in 100,000) abolishes or reduces active relaxation of circular fibres of frog's stomach by adrenaline (Table II). Glucose, 0·1 p.c., restores the response.

TABLE I. *The effect of adrenaline* (1 *in* 40,000) *on active relaxation of frog's stomach muscle. The stomach was divided transversely into* 5 *equal parts, the first part including a bit of the œsophagus and the cardio-œsophageal sphincter. Temperature* 27·5° *C.*

No. of experiment	Active relaxation ; p.c.				
	Part of stomach				
	1	2	3	4	5
1	6	14	5	0	0
2	22	21	17	18	0
3	28	25	26	22	20
4	20	50	38	34	0
5	34	6	24	6	7
6	20	18	12	12	0
7	12	26	33	17	25
8	22	25	25	14	8
9	23	43	27	27	22
10	14	25	19	14	9
11	23	36	36	18	0
12	23	17	21	11	6
13	12	25	36	22	14
14	20	23	34	34	30
15	40	31	22	12	14
16	0	20	25	19	7
17	36	19	27	30	8
18	7	27	20	21	0
19	7	33	44	44	0
20	16	22	20	20	0
Average ..	19	25	26	20	9

TABLE II. *Effect of sodium cyanide* (1 *in* 100,000) *and* 0·1 *p.c. glucose on active relaxation of circular muscle of frog's stomach by adrenaline* (1 *in* 40,000) *Temperature* 24·5° *C.*

No. of experiment	Active relaxation ; p.c.		
	In saline	In cyanide	In cyanide +glucose
1	26	0	13
2	34	6	29
3	28	0	21
4	24	0	10
5	13	0	11
6	29	0	24
7	31	0	10
8	44	0	16
9	12	0	10
10	7	0	23
11	17	0	0
12	22	0	6
13	30	0	12
14	36	0	22
15	7	0	10
Average ..	24	0·4	14

Effect of temperature.—The optimum temperature for active relaxation of frog's stomach muscle by adrenaline is 25–30° C. (Tables III and IV). Raising of the temperature above 35° C., permanently impairs active relaxation. Temperature is one of the most important items to be kept in mind when studying active relaxation. Too high or too low temperatures abolish active relaxation. Active relaxation in frog's stomach muscle undergoes seasonal variations; it is best obtained when the room temperature is from 25–30° C. During the winter months, it diminishes or is absent, even if the temperature of the saline is raised to 25–30° C.

TABLE III. *Effect of temperature on active relaxation of circular muscle of frog's stomach produced by adrenaline* (1 *in* 100,000)

Temperature °C.	Active relaxation ; p.c.						
	No. of experiment						Average
	1	2	3	4	5	6	
25	18	20	13	0	13	19	14
30	15	11	13	11	13	21	14
35	5	0	0	0	0	5	2
30	10	0	0	0	0	0	2

TABLE IV. *Effect of temperature on active relaxation of circular muscle of frog's stomach produced by adrenaline* (1 *in* 100,000)

Temperature °C.	Active relaxation ; p.c.						
	No. of experiment						Average
	1	2	3	4	5	6	
25	28	22	17	30	22	35	26
20	4	0	0	11	5	0	3
25	22	22	9	17	28	25	21

Effect of ions.—Substances which produce tonic contraction abolish active relaxation. Treatment with such substances permanently impairs active relaxation, this was tested by replacing 50 p.c. of the sodium of the

saline with potassium (15 experiments) and all the sodium chloride of the saline with iso-osmotic quantity of barium chloride (Table V).

TABLE V. *Effect of barium chloride on active relaxation of circular muscle of frog's stomach produced by adrenaline* (1 *in* 40,000). *Temperature* 27·5° *C. Sodium chloride of the saline replaced with iso-osmotic barium chloride*

No. of experiment	Active relaxation; p.c.		
	In saline	In barium	In saline
1	25	0	0
2	40	0	11
3	28	0	5
4	28	0	0
5	21	0	0
6	24	0	6
7	50	0	5
8	29	0	0
9	21	0	0
10	40	0	0
11	31	0	0
12	33	0	6
13	25	0	0
14	30	0	6
15	15	0	7
Average ..	29	0	3

TABLE VI. *Effect of hydrogen ions on active relaxation of circular muscle of frog's stomach produced by adrenaline* (1 *in* 40,000). *Temperature* 24° *C.*

No. of experiment	Active relaxation; p.c.		
	pH 8	pH 6·5	pH 8
1	25	13	13
2	11	10	20
3	22	5	16
4	24	9	16
5	20	0	10
6	44	0	5
7	32	0	9
8	21	0	9
9	30	9	9
10	23	5	22
11	0	0	14
12	50	3	11
13	23	0	11
14	23	3	8
15	27	0	20
Average ..	25	4	13

Substances that make the muscle inexcitable, also abolish active relaxation, such as excess of ammonium, potassium, iodide, thiocyanate.

Hydrogen ions diminish active relaxation (Table VI). At pH 5 active relaxation is abolished. In previous paper (Singh and Singh, 1950 *a*) active relaxation was better obtained at pH 6·5 than at pH 8. This was due to the fact that those experiments were performed at 20° C., at which temperature tone increases and thus active relaxation is antagonised. The effect of hydrogen ions was thus an indirect one, improvement being due to reduction of tone.

Hydrogen ions ordinarily cause relaxation of unstriated muscle. This relaxation has been found to be active. Ordinarily active relaxation was not produced by hydrogen ions, but after treatment with adrenaline and subsequent washing, the muscles became sensitive to hydrogen ions. Raising the acidity from pH 8 to 6·5, caused the circular muscle of frog's stomach to relax actively in 15 experiments at 23·5° C., the elongation being 26, 20, 21, 10, 6, 11, 25, 35, 22, 13, 20, 26, 33, 33 and 30 p.c. respectively.

Increase of osmotic pressure of the saline is also known to cause relaxation of unstriated muscle; this has been found to be active. Thus in 8 experiments, active relaxation of frog's muscles was 6, 13, 13, 20, 20, 5, 20 and 13 p.c. respectively, when the osmotic pressure of the saline was raised to 1·4 times normal by adding sodium chloride.

Increase of osmotic pressure of the saline decreases the active relaxation produced by adrenaline (1 in 40,000). Thus active relaxation in 6 frog's muscles at normal osmotic pressure was 21 $\pm$ 6 p.c.; in saline, the osmotic pressure of which was raised by 40 p.c. by adding sodium chloride, it was 5 $\pm$ 4 p.c.

Ammonium produces relaxation of frog's stomach muscle at pH 7 (Singh, 1939). This relaxation has been found to be active. Thus in 8 experiments, the active increments in length were 5, 0, 0, 11, 13, 5, 11 and 11 p.c. respectively at 20° C.; the optimum temperature is, however, 25-30° C.

Virgin guinea pig's uterus (Singh and Singh, 1951) is very sensitive to adrenaline; 1 in 1000 million may cause it to relax actively by about 25 p.c. Maximum relaxation is caused by 1 in 5 to 10 million. Large concentrations (1 in 50,000) first cause relaxation and then contraction. The muscle may relax spontaneously. The optimum temperature for active relaxation is 25 to 30° C.

Discussion

Active relaxation of unstriated muscle by adrenaline shows that tonus of unstriated muscle can be divided into three categories: (1) The muscle does not relax either actively or passively. (2) The muscle relaxes actively as well as passively. (3) The muscle relaxes passively but not actively.

If it is considered that the twitch contraction is due to the lactic mechanism, then only the alactic tonic contraction should relax actively. But twitch contraction has been shown to relax actively also. This suggests that the contractile mechanisms of the two systems of unstriated muscle are arranged in parallel; when one contracts, the other also contracts. Therefore normally there must be an inhibitor as well as an activator of the alactic mechanism.

When the muscle contracts, activation of the alactic mechanism would lead to slow relaxation, otherwise its inhibition would lead to active relaxation. What happens when the muscle only relaxes passively? In this instance, active relaxation must also be inhibited. The interrelation between these various reactions has been shown previously (Singh and Singh, 1949 *a*).

In unstriated muscle, therefore, there must be at least three substances concerned in the working of the system, in which energy for contraction is derived from that previously stored in the structure. These are: (1) Activator of the alactic mechanism which causes slow relaxation. (2) Inhibitor of the same, which inhibits slow relaxation, but produce active relaxation. (3) Inhibitor of active relaxation. It is possible, therefore, the mechanism which derives energy for contraction from the chemical stores, is evolved from the other one by combination of the latter with an inhibitor. When the energy stores are depleeted, this later mechanism may be converted into the earlier one, that is one which derives its energy from that stored in the structure, just as the gasping centre becomes dominant on failure of respiration, thus accounting for the *rigor mortis* of striated muscle.

Active relaxation due to the action of adrenaline on the contractile mechanism should be tested on a background of milk contraction, such as produced by pH 6·5, phosphate (Singh and Singh, 1950 *d*); there are individual and seasonal variations, some muscles not contracting in phosphate. Similarly, the action of acetylcholine on the contractile mechanism should be tested on a background of relaxation, such as is produced by potassium (Singh and Singh, 1949 *b*).

Summary

(1) Adrenaline produces active relaxation of the circular muscle fibres of frog's stomach.

(2) Longitudinal fibres show little active relaxation.

(3) Maximum elongation of the circular fibres of frog's stomach is caused by adrenaline, 1 in million.

(4) The middle portions of frog's stomach are most sensitive to adrenaline; the pyloric potion is the least sensitive.

(5) Cyanide abolishes active relaxation of frog's stomach muscle by adrenaline; this is restored by glucose.

(6) The optimum temperature for active relaxation of frog's stomach muscle by adrenaline is 25–30 °C.

(7) Substances that produces tonic contraction antagonise active relaxation.

(8) Substances that cause loss of excitability also abolish active relaxation.

(9) Acidity diminishes active relaxation.

(10) Hydrogen ions cause active relaxation.

(11) Increase in osmotic pressure of the saline to 1·4 times normal, causes active relaxation.

(12) Increase of osmotic pressure of the saline to 1·4 times normal diminishes active relaxation by adrenaline.

(13) Rabbit's gut muscle is not relaxed actively by adrenaline.

(14) Ammonium produces active relaxation of frog's stomach muscle.

References

Hill, A. V. .. *Proc. Roy. Soc.*, 1949, **136B**, 420.

Kaiser, K. .. *Zbl. Physiol.*, 1900, **14**, 195, Quoted from Hill, A. V., *Proc. Roy. Soc.*, 1949, **136B**, 420.

Kuhne .. *Arch. Anal. Physiol.*, 1859, 815. Quoted from Hill, A. V., *Proc. Roy. Soc.*, 1949, **136B**, 420.

Ramsey, R. W. and Street, S. F. .. *J. Cell. Comp. Physiol.*, 1940, **15**, 11, Quoted from Hill, A. V., *Proc. Roy. Soc.*, 1949, **136B**, 420.

——— .. *Biol. Symp.*, 1941, **3**, 9, Quoted from Hill, A. V., *Proc. Roy. Soc.*, 1949, **136B**, 420.

Singh, I. .. *J. Physiol.*, 1939, **96**, 367.

Singh, S. I. and Singh, I. .. *Curr. Sci.*, 1948, **17**, 306.

——— .. *Proc. Ind. Acad. Sci.*, 1949 *a*, **30**, 343.

——— .. *Ibid.*, 1949 *b*, **30**, 270.

——— .. *Curr. Sci.*, 1950 *a*, **19**, 60.

——— .. *Nature*, 1950 *b*, **166**, 647.

——— .. *Proc. Ind. Acad. Sci.*, 1950 *c*, **31**, 351.

——— .. *Ibid.*, 1950 *d*, **32**, 12.

——— .. *Curr. Sci.*, 1951, **20**, 48.

AN EMBRYOLOGICAL STUDY OF *MIMOSA PUDICA* LINN.

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In a previous paper by the writer (Narasimhachar, 1948) an embryological study of *Acacia farnesiana* was given. The material selected for the present investigation is *Mimosa pudica*, Linn. another member of Mimosaceæ. This hardy perennial plant seems to have been introduced from America and has spread now as a weed throughout the Mysore State in both waste, as well as cultivated lands. Having a well-developed root system, it is extremely drought-resistant and perennates during the summer. The plant is gregarious in habit and has a wide dispersal mechanism. It is not easy to eradicate this pest once it has established a foot-hold in the soil. The best method of eradication seems to be to dig out the entire plant with the roots before it sets seeds. Specially during the rainy season the plants may be easily spotted out by their pink flowers contrasting against a back ground of rich green foliage.

Buds, flowers, and fruits were fixed in Formalin-acetic-alcohol and Allen's modified Bouin's fluid. Sections were cut from 6μ to 10μ and stained in Heidenhain's Iron-alum Hæmotoxylin.

Microsporangium

The wall of the young anther shows three layers beneath the epidermis-the endothecium, a middle layer, and the innermost layer or the tapetum (Fig. 1). The endothecium develops fibrillar thickenings when the anther is mature, and the middle layer is crushed. The tapetal cells remain uninucleate throughout (Fig. 2). In the early stages during the course of development, sterility of sporogenous tissue is invariably seen due to failure of meiotic stages (Fig. 3). The microspore mother-cells undergo the usual reduction divisions and form microspore quartets which show either a bilateral or tetrahedral arrangement (Figs. 4 to 8). The individual microspores of the quartet do not separate but remain as a unit and are shed in the same condition as seen in the compound grains of *Acacia Baileyana* (Newman, 1934), *Acacia farnesiana* (Narasimhachar, 1948) and *Drosera Burmanni* (Narasimhachar, 1949). Each mature pollen grain contains a generative and a tube nucleus (Fig. 9). Germination of pollen grains *in*

situ was found in many anther locules (Fig. 2) suggesting a tendency towards cleistogamy.

During the study of meiosis in the microspore mother cells, 24 bivalents were counted (Fig. 4) in the first metaphase plate which confirms the $2x$ number 48 reported by Kawakami (1930).

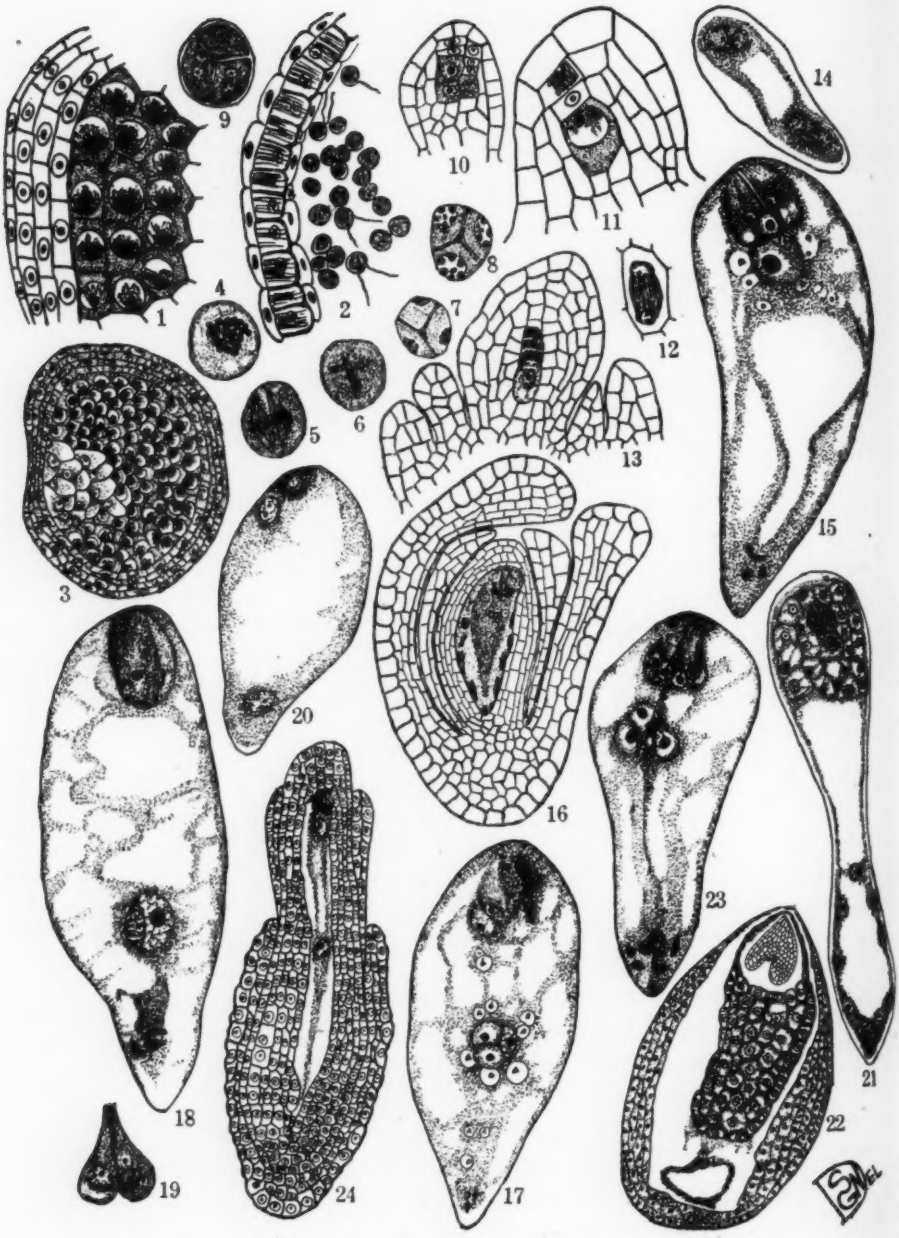
Megasporogenesis and the Development of the Embryosac

The superior unilocular ovary develops three to five ovules on the marginal placenta. The ovule is at first erect but later on becomes anatropous due to subsequent growth. The archesporium consists of three to four cells (Fig. 10) hypodermal in origin. Only a single cell develops further (Fig. 11) and cuts off parietal cells, so that the megaspore mother-cell is situated about 3–4 cell layers below the epidermis. A multiple epidermis is later on developed as in *Acacia Baileyana* (Newman, 1934).

The megaspore mother-cell crushes the surrounding nucellar cells as it enlarges in size and after undergoing two successive divisions gives rise to the linear tetrad (Fig. 11) of megaspores. The chalazal megaspore enlarges in size and forms the typical monosporic eight nucleate embryosac, while the upper three megaspores degenerate.

The mature embryosac is broad at the micropylar end and tapers towards the chalazal end (Fig. 15). All the parietal tissue later on gets obliterated by the developing embryosac so that it lies next to the multiple epidermis. The cells of the multiple epidermis break down as the embryosac enlarges in size contributing to the nutrition of the embryosac. The antipodals are organized as definite cells and show signs of degeneration when the embryosac is fully organized, and the degenerating antipodals may persist until fertilization (Figs. 15 to 18). The synergids are pyriform and slightly beaked with the filiform apparatus (Fig. 16). A vacuole is situated beneath the nucleus in each synergid. The position of the egg is variable. It may be situated at one side of the synergid or between the synergids. The two polars may remain in contact with each other or may fuse to form a big fusion nucleus when the embryosac is ready for fertilization (Figs. 15 and 16), and is found to lie immediately beneath the egg, or in the middle of the embryosac. Starch grains are found in the embryosac from the eight-nucleate stage onwards and persist as in *Acacia Baileyana* (Newman, 1934), and *Acacia farnesiana* (Narasimhachar, 1948).

Occasionally two embryosacs have been met with probably due to two megaspore mother-cells developing simultaneously. This is considered to be an abnormality, as these embryosacs are found to degenerate later (Fig. 23).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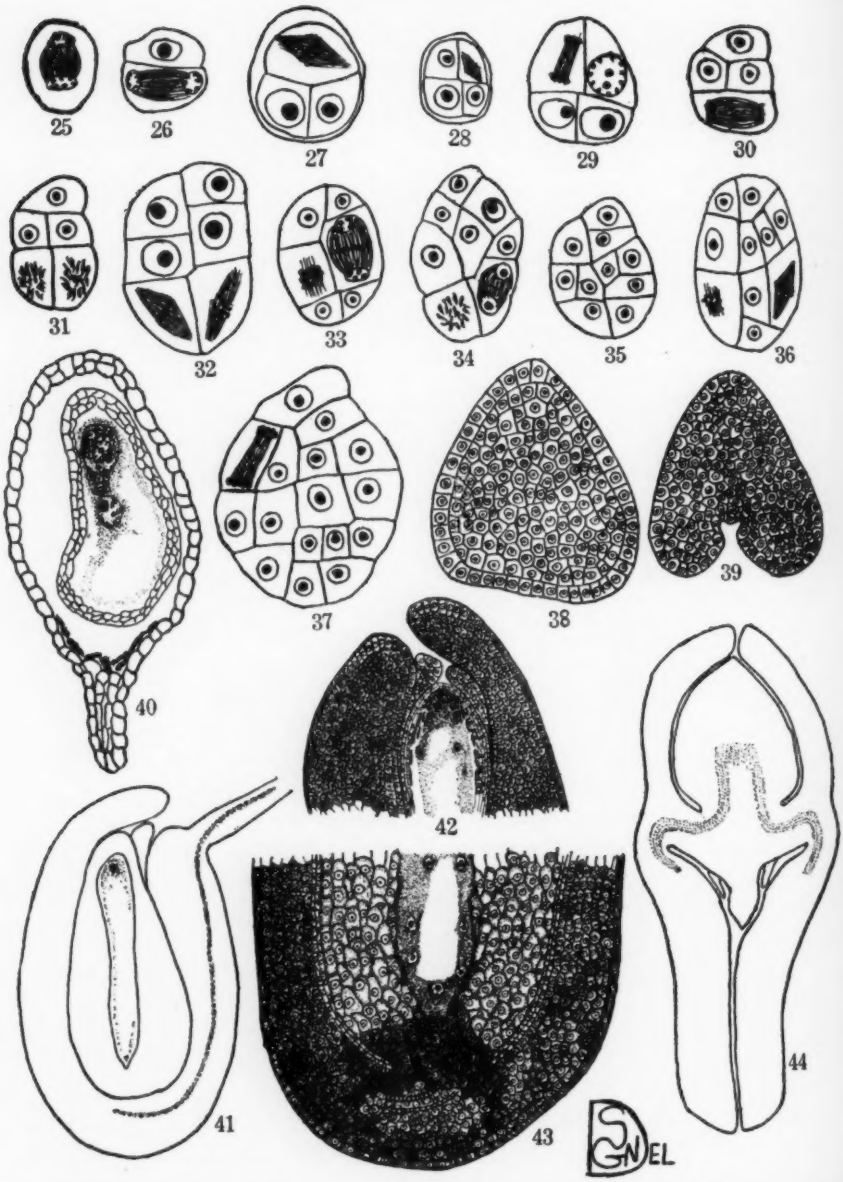
FIGS. 1–24. Fig. 1. Portion of a transverse section of young anther showing epidermis, wall layers, tapetum and microspore mother-cells, × 480. Fig. 2. Portion of the mature anther showing the prominent fibrillar endothecium and uni-nucleate tapetum. Some of the microspore quartets are germinating *in situ*, × 800. Fig. 3. Portion of a transverse section of young anther showing degenerating sporogenous tissue and normal sporogenous cells, × 400. Fig. 4. Metaphase plate showing 24 bivalents, × 3,600. Figs. 5 & 6. Spindle arrangement to form isobilateral or tetrahedral microspore quartets, × 3,600. Figs. 7 & 8. Stages in the formation of microspore tetrads, × 3,600. Fig. 9. A quartet of pollen grains at the shedding stage, each showing a generative and a tube nucleus, × 3,600. Fig. 10. Multiple archesporium, × 1,800. Fig. 11. Megaspore mother-cell and the parietal cell dividing, × 1,800. Fig. 12. Telophase in the megaspore mother-cell to form the diad, × 1,800. Fig. 13. Linear tetrad showing the enlarging chalazal megaspore and the degenerating upper three megaspores, the parietal layer and the integuments, × 1,080. Fig. 14. Division of the nuclei in the two nucleate embryosac to form the four-nucleate embryosac, × 1,800. Fig. 15. Fully organized embryosac showing the egg-apparatus, Secondary nucleus, degenerating antipodals and starch grains, × 1,800. Fig. 16. Longitudinal section of an ovule showing the fully organized embryosac with the egg apparatus, polars, degenerating antipodals and starch grains with the fully formed integuments, × 1,800. Figs. 17 & 18. Stages in double fertilization with the male nuclei in contact with the egg and the Secondary nucleus respectively, × 1,800. Fig. 19. Egg-apparatus with synergids × 1,800. Fig. 20. Fertilized egg with two endosperm nuclei, × 1,800. Figs. 21 & 22. An advanced embryo with cellular endosperm around the egg and lower portion showing nuclear endosperm, × 1,800. Fig. 23. Abnormal ovule with two embryosacs, × 1,800. Fig. 24. Longitudinal section of an ovule showing ill-formed outer integument and degenerating zygote, × 480.

The integuments are rather belated in appearance. The inner integument appears first and later the outer integument. By the time the mature embryosac is organised, the inner integument covers the nucellus completely excepting the micropyle, while the outer integument overgrows the inner one (Fig. 16). The inner integument is only two cells thick except at the micropyle while the outer one is three to four cells in thickness, with a single vascular strand entering from the funiculus (Fig. 41). It becomes more massive after fertilization crushing the cells of the inner integument at the sides of the embryosac except at the micropyle (Figs. 42 and 43). Occasionally in degenerating embryosacs the outer integument may develop only halfway while the inner integument overgrows it (Fig. 24).

During fertilization, one of the synergids is destroyed by the entry of the pollen tube while the other one persists for a long time. Both syngamy and triple fusion occur as a normal process during fertilization (Figs. 17 and 18).

ENDOSPERM

The primary endosperm nucleus divides much earlier than the fertilized egg in a free nuclear manner (Fig. 20). By this time, the embryosac has increased in size. This is accompanied by the increase in number of endosperm nuclei which take up a peripheral position lining the embryosac. Some nuclei cluster round the developing zygote and others at the chalazal end.



FIGS. 25–44. Figs. 25–30. Stages in embryogeny. (Figs. 25–27 × 1,800) (Figs. 28–30 different ways of forming the five-celled embryo, × 1,800). Fig. 40. Longitudinal section of an ovule showing the zygote in Telophase, four hypertrophied endosperm nuclei and two

abnormal endosperm nuclei. Starch grains still persisting, × 1,800. Fig. 41. Longitudinal section of ovule showing the disposition of the vascular strand, × 800. Fig. 42. Longitudinal section of an advanced ovule showing the massive outer integument and the crushed cells of the inner integument below the micropylar region, × 800. Fig. 43. Longitudinal section of an advanced ovule showing the chalazal hypostase like tissue, free endosperm nuclei in contact with disorganizing multiple epidermis and the chalazal region with the ingrowing embryosac containing free endosperm nuclei, × 800. Fig. 44. A longitudinal section of a mature seed showing the dicotyledonous embryo, with the spurred cotyledons, stem and root tip, × 100.

Cell formation is restricted to the upper two-thirds of the embryosac while the lower end consists of free endosperm nuclei (Figs. 21 and 22) as in *Acacia Baileyana* (Newman,1934) and *Acacia farnesiana* (Narasimhachar, 1948). At the micropylar end of the embryosac most of the parietal tissue and multiple epidermis are destroyed (Fig. 42) while at the lower end, the embryosac with the free endosperm nuclei shows a tendency to grow into the chalazal region consisting of cells rich in contents (Fig. 43) simulating the basal apparatus of *Hypericum* (Swamy, 1946) but without developing into any special organ of the endosperm. In addition, some layers of the multiple epidermis bordering the sides of the embryosac break down. Thus the endosperm tissue and the basal apparatus with free endosperm nuclei seem to take part in providing nutrition to the growing embryo.

Instances are occasionally met with in degenerating embryosacs, hypertrophied and normal endosperm nuclei, as in Fig. 40 where the fertilized egg is in telophase with four hypertrophied and two normal endosperm nuclei.

Embryo

The first division of the zygote does not take place until sixteen endosperm nuclei are formed, while in *Acacia Baileyana* (Newman, 1936) and *Albizzia Lebbeck* (Maheshwari, 1931) the zygote does not divide until eight endosperm nuclei are formed, in *Acacia farnesiana* (Narasimhachar, 1948) the zygote divides at the thirty-two nucleate stage of the endosperm. The first division of the fertilized egg is transverse (Fig. 25) dividing it into an upper and a lower cell. The second division takes place in the lower cell (Fig. 26) much earlier than the upper cell so that the embryo consists of four cells. From the four-celled stage onwards the divisions are irregular (Figs. 25 to 37) as any cell in the four-celled embryo may divide to give rise to the five-celled stage of the embryo (Figs. 28 to 30). Soon the embryo assumes a pear-shaped form. There is no differentiation into a suspensor and the embryo is of the massive type as in *Acasia Baileyana* (Newman, 1936) and *Acacia farnesiana* (Narasimhachar). In the mature seed the embryo uses up all the endosperm. The cotyledons are prominently spurred with vasculation on the adaxial face (Fig. 44). The radicle is very broad and blunt.

SUMMARY

1. The pollen grains are shed united together in quartets. Each pollen grain contains a generative and a tube nucleus. The tapetum remains uni-nucleate throughout. During the first metaphase, 24 bivalents have been counted.

2. The archesporium consists of three to four cells though only a single cell develops further. A parietal tissue of 4–5 cells in thickness is formed.

3. Megasporogenesis proceeds normally and the embryosac conforms to the monosporic eight-nucleate type. Synergids show the characteristic filiform apparatus and the polars may fuse before or during fertilization. Antipodals are formed as definite cells, and the degenerated remains may persist until fertilization.

4. Endosperm is free nuclear in the beginning and wall formation commences from the micropylar end stopping short of the lower one-third of the embryosac. The chalazal end contains free endosperm nuclei which aggregate simulating the basal apparatus with a tendency to grow into the chalazal region.

5. The first division of the fertilized egg is transverse while the second division which is vertical is belated in the upper cell. Later divisions are irregular and the embryo has no differentiated suspensor, being of the massive type.

In conclusion, grateful acknowledgements are made to Dr. L. S. Dorasami, M.Sc., PH.D. (Lond.), Economic Botanist, for kind encouragement during the course of the work.

LITERATURE

1. Guignard, L. .. "Recherches d'embryogenien vegetale comparee: 1. Legumineuses," *Ann. Sci. Nat. Bot. Ser.*, 1881, 6-12, 125–66.
2. *Kawakami, I. .. *Bot. Mag. Tokyo*, 1930, **44**, 319–28.
3. Maheshwari, P. .. "Contributions to the morphology of *Albizzia Lebbeck*," *Jour. Ind. Bot. Soc.*, 1931, **10**, No. 4, 241–64.
4. Narasimhachar, S. G. .. "A contribution to the morphology of *Acacia farnesiana* L. (Willd.)," *Proc. Ind. Acad. Sci.*, 1948, **28B**, 144–49.
5. ———— .. "A contribution to the embryology of *Drosera Burmannii* Vahl.," *ibid.*, 1949, **29B**, 98–104.
6. Newman, I. V. .. "Studies in the Australian Acacias: IV. The life-history of *Acacia Baileyana* F.V.M. Part II. Gametophytes Fertilization, seed production and germination and general conclusions," *Proc. Linn. Soc.*, New South Wales, 1934, **59**, 277–313.
7. Schnarf, K. .. *Vergleichende Embryologie der Angiospermen*, Berlin, 1931.
8. Swamy, B. G. L. .. "Endosperm in *Hypericum mysorense* Heyne," *Annals of Botany*, April 1946. N. S., **10**, No. 38.

* Citation from '*Chromosome Atlas of Cultivated Plants*,' by Darlington, C. D., and Janakiammal, E. K., George Allen & Unwin, Ltd., London, 1945.

STUDIES IN FISH LIVER OILS

Part III. Biological Assay of Vitamin D

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Indian Shark liver oils are now in use as substitutes for cod-liver oil. It is well known that cod-liver oil is rich in both vitamins A and D. Ranganathan (1941), Niyogi, Patwardhan and Acharya (1943) working on the vitamin content of some of the Indian fish liver oils have come to the conclusion that whereas their vitamin A content is high, their vitamin D is almost negligible. Also Das, Acharya and Devadatta (1949) working on the biological assay of vitamin A of some of the liver oils of fish available at Bombay have shown that these oils contain large quantities of vitamin A. If cod liver oil is to be replaced by Indian fish liver oils, the latter must contain in addition to vitamin A, sufficient quantities of vitamin D. Since the previous authors Ranganathan (*loc. cit.*) and Niyogi, *et al.* (*loc. cit.*) have not investigated all the species of fish available in Indian coastal waters, it was thought fit to investigate the vitamin D content in the liver oils of some species of fish.

The present work therefore deals with the estimation of vitamin D content of six varieties of fish liver oils investigated by Das, Acharya and Devadatta (*loc. cit.*) for their content of vitamin A.

Experimental

The six liver oils that were used for the estimation of vitamin D were extracted by the process described by Das, Acharya and Devadatta (1949). Fresh livers were minced and mixed with twice their volume of water in a tinned copper vessel. The contents of the vessel were cooked in a water-bath with constant stirring and keeping the temperature at about 80° C. The oil that oozed out was collected, filtered through muslin cloth and transferred to a Winchester bottle. The crude oil thus collected was allowed to settle for an hour to remove the water layer. To remove the traces of moisture, the oil was again transferred to the bottle, gently warmed in a water-bath and a small quantity anhydrous sodium sulphate was added. The contents of the vessel were shaken vigorously and kept overnight in

the presence of nitrogen atmosphere. The clear oil was stored in a coloured bottle in the presence of inert atmosphere.

Biological Estimation of Vitamin D

Coward (1939) has stated that the most accurate of the methods in general use for the determination of vitamin D content of a substance is 'the bone ash method'. Hence the bone ash method was adopted in this investigation.

Since the oils under investigation contained very large quantities of vitamin A, it was likely that it might interfere with the vitamin D assay. The vitamin A was therefore destroyed by passing a current of oxygen through the oil which was maintained at 100° C. When a sample of the oil thus treated failed to give blue colour with antimony trichloride dissolved in chloroform, the bubbling of oxygen was stopped; this process took about two hours in this investigation. According to Drummond and Coward (1920) complete destruction of vitamin A could be had by passing air at 96° C. for three hours.

The diet used in these experiments is principally the rachitogenic diet of Schneider and Steenbock (1939). This diet is adequate in calcium but low in phosphorus content. The same diet was successfully used by Niyogi, *et al.* (*loc. cit.*) for the estimation of vitamin D in some fish liver oils.

Composition of the diet.—The vitamin D free basal diet had the following composition:

	Parts
Cane Sugar ..	49·00
Starch ..	21·5
Egg White (dried) ..	18·00
Sodium Chloride ..	1·00
Calcium Carbonate ..	1·50
Marmite ..	4·00
Olive Oil ..	4·50
Red Palm Oil ..	0·50

There are two points in favour of using the above diet in the vitamin D assay work: (*a*) a greater measure of success is assured in producing rickets in rats and (*b*) the experimental period is shortened.

Procedure

Fifty-six young rats four weeks old and weighing between 35 and 40 grams were equally divided into seven groups eight in each and fed with the

rachitogenic diet. The examination of epiphyses of tibiæ of these animals showed presence of rickets. After creating rickets in these rats which took ten to twelve days, three groups out of the seven were given orally a supplement of 3·6, 7·2 and 14·4 mgm. of Khada mushi liver oil per rat per week, for three weeks. The animals in the other three groups, *i.e.*, the control groups (for the standard oil) received doses of 0·175 I.U. 0·35 I.U. and 0·7 I.U. of Vitamin D respectively of the standard oil per rat per week for three weeks. One group served as the control which did not receive either the standard oil or the Khada mushi liver oil. The assay period lasted for three weeks at the end of which the rats were killed, their femora removed and freed as completely as possible from their adhering tissue by washing with water and rubbing with a piece of cheese cloth. Each bone was broken into two, tied up in a piece of filter-paper and treated with a large volume of boiling alcohol for about an hour and then with ether for at least twenty-four hours in a soxhelet extractor. The bones were then dried to a constant weight. Each pair of bones was ashed by heating it in a silica crucible over a bunsen burner to a constant weight and the ash content determined. The average of the results given by the animals in each group was taken and compared. The same procedure was adopted in the case of other fish liver oils.

For calculating the vitamin D content, the percentage weight of ash was plotted against the logarithms to the base ten, of the doses given (milligrams of the unknown or the International Units of the Standard oil). The value for vitamin D was calculated from the graph by finding out the doses for the same increase in weight of the standard oil and the unknown, following the method adopted by Coward (*loc. cit.*) as well as, by calculating directly by the rule of three method. The results of the assay are given in Table II below.

TABLE II

The dose and the percentage increase in weight of bone ash for six different varieties of fish liver oils as compared with the standard cod liver oil in the determination of the vitamin D content of the former.

(Results are given for complete three weeks test period)

Rats on experimental oil		Rats on standard cod liver oil				
Dose of oil in mg.	% of ash	Dose of Std. oil in I. U.	% of ash	I. U. of Vit. D./gm. of oil by calculation	Average I. U. of Vit. D./gm. of oil by calculation	I. U. of Vit. D by graph
Oil No. 1. Local name of fish :—Khada mushi or Kodaicha (*Calcarius melanopterus*)						
..	28·60	..	28·60	..	..	..
3·6	31·48	0·5250	31·34	157·5	..	..
7·2	34·26	1·0500	34·38	147·0	150·2	138·6
14·4	39·10	2·1000	39·56	146·0	..	..
Oil No. 2. Local name :—Waghbeer or Waghsbeer (*Galeocerasfigrinus*)						
..	29·10	..	29·10	..	..	..
3·6	31·61	0·5250	31·65	143·5	..	..
7·2	33·98	1·0500	34·20	139·6	140·7	114·8
14·4	38·78	2·1000	39·25	139·1	..	..
Oil No. 3. Local name :—Kan Mushi (*Cestracion Blochii*)						
..	29·51	..	29·51	..	..	..
3·6	31·36	0·5250	32·11	103·8	..	..
7·2	33·15	1·0500	34·35	104·0	104·7	108·6
14·4	36·80	2·1000	39·76	106·4	..	..
Oil No. 4. Local name :—Nali, Win Sonder or Khandere (*Pristis Perrotteti*)						
..	28·85	..	28·85	..	..	..
3·6	30·95	0·5240	31·60	111·3	..	..
7·2	33·20	1·0500	34·81	106·4	108·2	114·8
14·4	37·50	2·1000	39·65	..	..	..
Oil No. 5. Local name :—Wagli (*Rhinoptera Javanica*)						
..	29·25	..	29·25	..	..	..
3·6	31·20	0·5250	31·95	..	..	..
7·2	33·23	1·0500	34·85	105·3	102·6	106·8
14·4	37·25	2·1000	40·25	97·2	..	..
Oil No. 6. Local name :—Surmai (*Cybium Kuhlii*)						
..	28·95	..	28·95	..	..	..
3·6	31·05	0·0500	31·75	107·5	..	..
7·2	32·95	1·0500	34·45	106·0	103·9	109·6
14·4	37·15	2·1000	40·15	97·9	..	..

SUMMARY AND CONCLUSION

The vitamin D content of the six liver oils studied, *viz.*, Khada Mushi, Waghbeer, Kan Mushi, Nali, Wagli and Surmai are found to be respectively 150·2, 140·7, 104·7, 108·2, 102·6 and 103·9 I.U. per gram of oil. The assay reveals that in comparison to their vitamin A content the vitamin D

content is almost negligible, a finding which agrees with that of Ranganathan (*loc. cit.*) and Niyogi, Patwardhan and Acharya ((*loc. cit.*).

References

1. Coward .. .. *The Biological Standardisation of Vitamins*, Baillier Tindall and Cox, London, 1938.
2. Das, B. R. S., Acharya, B. N. and Devadatta, S. C. *Proc. Ind. Acad. Sci.*, 1949, **29**, 13.
3. ———— .. .. *Ibid.*, 1949, **30**, 299.
4. Drummond and Coward .. *Biochem. Jour.*, 1920, **14**, 734.
5. Niyogi, S. P., Patwardhan, V. N. and Acharya, B. N. *Ind. Jour. Med. Res.*, 1943, **31**, 15.
6. Ranganathan .. .. *Ibid.*, 1941, **29**, 699.
7. Schneider and Steenbock .. *Jour. Biol. Chem.*, 1939, **128**, 159.

THE NON-SUGARS IN SUGARCANE JUICE AND THEIR BEARING ON CLARIFYING PROPERTIES: A STUDY IN RELATION TO THE OPEN PAN INDUSTRY

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I. Introduction

The importance of non-sugars in sugarcane juice and their bearing on clarifying properties are now recognised in vacuum pan practice. Kortscher (1939) discusses the adverse effects of different categories on non-sugars (such as pectin, pentosans, waxes, fats, polyphenols and non-albuminoid nitrogen compounds) present as "inherent colloids" in juice. Similarly, Alvarez (1938) associates superior factory quality with smaller gum and albuminoid contents while Hornby and Kasjanov (1939) state that the solubility of sucrose is increased by organic and inorganic "impurities". The so-called "harmful" or non-protein nitrogen compounds (Deerr, 1921; Browne and Zerban, 1941), which include amino acids, amides, organic bases, etc., are regarded as particularly injurious to manufacturing qualities. Soragato (1936) considers soluble nitrogen compounds to be so troublesome that he advocates evolving breeds of low nitrogen content. According to Ness (1941), these substances not only produce coloration but lower sugar recoveries as well. Apart from organic non-sugars, mineral constituents are also believed to exert undesirable influences. Thus, Alvarez (*loc. cit.*) considers low lime and magnesia contents as desirable juice criteria. Parashar, Kripashankar and Lal (1947) attribute various evil effects to inorganic constituents like silica, iron and alumina, lime and magnesia. While the role of silica in clarification is not fully understood, David, Gomez and Boon (1938) state that it exists in two forms—soluble silicates and complex organo siliceous compounds. Muller (1921) also recognises the existence of silica in juice in the form of an organic colloidal complex. Davis (1939, 1940) observes that organic silica per cent. total solids is inversely proportional to juice "claribility". The role of phosphates in juice clarification has engaged considerable attention in connection with the manufacturing difficulties experienced with the variety POJ 2878. These have been

connected with phosphate deficiency in some cases (Parashar, Kripashankar and Lal, *loc. cit.*; McCleery, 1933; Hadfield, 1934; Fleshman, 1936; Caminha, 1935; Davis, 1937; Springer, 1937), though not always explained on this basis (Davis, Duncan and Yearwood, 1936; Davis, 1939; Pocy, 1934; Guillaume, 1939; Birkett, 1940). The apparent anomalies have been sought to be explained by the postulation of effective or available phosphates, such as phosphates present as phosphoric acid (Beater, 1937) or phosphates in true solution (Gundu Rao and Kripashankar, 1939). Davis (*loc. cit.*) discovers a characteristic phosphate status in the clarified juice of a variety (leading to possibilities of organo-phosphates) and concludes that a better criterion is the residual phosphate level or the amount of phosphates eliminated.

The part played by non-sugars under the drastic treatments of the white sugar factory being thus clear, they may well be expected to assume even greater importance in the relatively inefficient open pan system of manufacture. In fact, large varietal differences in gur quality are commonly experienced, marked variation in respect of non-sugars in juices having also been observed (Ramanaya and Vishwanath, 1935; Ramanaya, 1936; Ramanaya and Satyanarayana, 1938; Ramanaya and Narasingha Rao, 1942; Parashar, 1942). It, therefore, becomes important to evaluate varieties for the open pan industry with regard to the nature and extent of non-sugars in their juices as also to assess the role of these ingredients in determining quality of the product. Some preliminary work already reported by the authors (1949) led to the formulation of certain definite characteristics for juices associated with superior clarifying properties and high quality of product, *viz.*, (i) a low level of total colloidal matter, gums, pectin and ash content and (ii) high phosphates.

In the above context, further detailed studies were considered of interest and in the present investigations certain new aspects have been examined, the work consisting of a study of juices of different varieties in respect of the following criteria, as affecting clarifying properties and quality of product:—

(1) The distribution of nitrogen in protein and non-protein (or "harmful") forms.

(2) Composition of the colloidal fraction with respect to organic and inorganic components.

(3) Distribution of mineral matter in colloidal and molecular forms.

(4) Relative amounts of different inorganic constituents.

TABLE

Showing averages for juice constituents of different varieties with standard

(Except when stated otherwise, values are

1 Varieties / Characters	2 Co 313	3 Co 383	4 Co 453	5 Co 513	6 BO 3	7 BO 10	8 BO 11	9 BO 24	
1 Sucrose ..	19·94	17·75	18·37	18·73	18·42	18·46	19·30	19·15	±0·7537
2 Glucose ..	0·26	0·39	0·28	0·32	0·42	0·34	0·50	0·32	±0·4169
3 Ash ..	0·57	0·69	0·86	0·56	0·67	0·68	0·67	0·67	±0·03
4 Saline coefficient	34·83	25·73	22·85	33·55	27·49	27·35	29·08	28·81	±1·7031
5 Total colloids	0·42	0·62	0·96	0·45	0·67	0·71	0·65	0·70	±0·0607
6 Ash per cent. in colloidal fraction	8·10	8·47	7·77	7·84	7·89	7·10	9·25	7·61	±1·19
7 Ash in colloidal fraction per cent. Total ash in juice	5·91	7·57	8·93	5·98	7·85	7·33	8·63	7·98	±0·60
8 Gums ..	0·088	0·127	0·150	0·090	0·133	0·133	0·119	0·133	±0·0072
9 Pectin (calcium pectate)	0·019	0·026	0·034	0·021	0·027	0·025	0·028	0·027	±0·002
10 Total organic nitrogen	0·073	0·070	0·070	0·078	0·074	0·069	0·070	0·068	±0·009
11 Non-protein nitrogen	0·0298	0·0418	0·0488	0·0313	0·0450	0·0468	0·0405	0·0425	±0·0059
12 Protein nitrogen	0·044	0·028	0·022	0·047	0·029	0·022	0·030	0·026	±0·0066

I

errors per observation and critical differences at 5 and 1 per cent. levels
expressed as per cent. on weight of juice)

10	11	12	13
S.E. per observation	C.D. at 5%	C.D. at 1%	Conclusions
±0·7537	1·0999	..	At 5%: Co 313 BO 11 BO 24 Co 513 BO 10 BO 3 Co 453 Co 383 Significant
±0·4169	..	..	Non-significant
±0·03	0·04	0·06	At 5%: Co 453 Co 383 BO 10 BO 3 BO 11 BO 24 Co 313 Co 513
			At 1%: Co 453 Co 383 BO 10 BO 3 BO 11 BO 24 Co 313 Co 513 Highly significant
±1·7031	2·48	3·36	At 5%: Co 313 Co 513 BO 11 BO 24 BO 3 BO 10 Co 383 Co 453
			At 1%: Co 313 Co 513 BO 11 BO 24 BO 3 BO 10 Co 383 Co 453 Highly significant
±0·0607	0·0888	0·1203	At 5%: Co 453 BO 10 BO 24 BO 3 BO 11 Co 383 Co 513 Co 313
			At 1%: Co 453 BO 10 BO 24 BO 3 BO 11 Co 383 Co 513 Co 313 Highly significant
±1·19	..	..	Non-significant
±0·60	0·87	1·17	At 5%: Co 453 BO 11 BO 24 BO 3 Co 383 BO 10 Co 513 Co 313
			At 1%: Co 453 BO 11 BO 24 BO 3 Co 383 BO 10 Co 513 Co 313 Highly significant
±0·0072	0·0211	0·0285	At 5%: Co 453 BO 3 BO 10 BO 24 Co 383 BO 11 Co 513 Co 313
			At 1%: Co 453 BO 3 BO 10 BO 24 Co 383 BO 11 Co 513 Co 313 Highly significant
±0·002	0·0029	0·0039	At 5%: Co 453 BO 11 BO 3 BO 24 Co 383 BO 10 Co 513 Co 313
			At 1%: Co 453 BO 11 BO 3 BO 24 Co 383 BO 10 Co 513 Co 313 Highly significant
±0·009	..	..	Non-significant
±0·0059	0·0087	0·0117	At 5%: Co 453 BO 10 BO 3 BO 24 Co 383 BO 11 Co 513 Co 313
			At 1%: Co 453 BO 10 BO 3 BO 24 Co 383 BO 11 Co 513 Co 313 Highly significant
±0·0066	0·0097	0·0131	At 5%: Co 513 Co 313 BO 11 BO 3 Co 383 BO 24 Co 453 BO 10
			At 1%: Co 513 Co 313 BO 11 BO 3 Co 383 BO 24 Co 453 BO 10 Higly significant

TABLE

1 Characters \ Varieties	2	3	4	5	6	7	8	9
	Co 313	Co 383	Co 453	Co 513	BO 3	BO 10	BO 11	BO 24
13 Non-protein nitrogen per cent. Total organic nitrogen	41·6	59·5	69·3	40·3	61·7	67·4	58·3	61·9
14 Soluble SiO_2	0·008	0·012	0·017	0·007	0·014	0·014	0·016	0·013
15 Fe_2O_3 + Al_2O_3	0·056	0·090	0·123	0·058	0·091	0·083	0·079	0·100
16 CaO ..	0·027	0·039	0·049	0·031	0·044	0·040	0·043	0·039
17 MgO	0·025	0·035	0·037	0·029	0·035	0·037	0·035	0·035
18 P_2O_5	0·030	0·022	0·021	0·030	0·024	0·023	0·022	0·021
19 Colour values	2406	3894	4510	2170	4456	4584	4576	3936

II. EXPERIMENTAL

The experiments were conducted with reference to eight varieties of cane (Cos. 313, 383, 453, 513 and BO's. 3, 10, 11, 24) in four replications. In each case, there were two series of pans boiled, *viz.*, (i) no treatment in juice and (ii) castor seed extract treatment (Khanna and Chacravarti, 1949). The conditions of boiling were carefully standardised, the same quantity of juice being boiled in all cases, using the same type of furnace and equipment (Khanna and Prasad, 1947). To examine the degree of reproducibility obtained in pan conditions, the rate of temperature rise was followed between 105° C. and 118° C., all pans being struck at the latter temperature.

In all cases, the juice was analysed for brix, sucrose (polarisation), glucose (reducing sugars), total colloids, gums, pectin, total organic nitrogen,

I—(*Contd*).

10	11	12	13
S.E. per observation	C.D. at 5%	C.D. at 1%	Conclusions
±4·87	7·12	9·65	At 5%: Co 453 BO 10 BO 24 BO 3 Co 383 BO 11 Co 313 Co 513
			At 1%: Co 453 BO 10 BO 24 BO 3 Co 383 BO 11 Co 313 Co 513 Highly significant
±0·0035	0·0052	0·0070	At 5%: Co 453 BO 11 BO 10 BO 3 BO 24 Co 383 Co 313 Co 513
			At 1%: Co 453 BO 11 BO 10 BO 3 BO 24 Co 383 Co 313 Co 513 Highly significant
±0·0161	0·024	0·032	At 5%: Co 453 BO 24 BO 3 Co 383 BO 10 BO 11 Co 513 Co 313
			At 1%: Co 453 BO 24 BO 3 Co 383 BO 10 BO 11 Co 513 Co 313 Highly significant
±0·0044	0·0064	0·0087	At 5%: Co 453 BO 3 BO 11 BO 10 Co 383 BO 24 Co 513 Co 313
			At 1%: Co 453 BO 3 BO 11 BO 10 Co 383 BO 24 Co 513 Co 313 Highly significant
±0·0087	..	..	Not significant
±0·003	0·0043	0·0059	At 5%: Co 313 Co 513 BO 3 BO 10 BO 11 Co 383 Co 453 BO 24
			At 1%: Co 313 Co 513 BO 3 BO 10 BO 11 Co 383 Co 453 BO 24 Highly significant
±381·61	556·89	754·66	At 5%: BO 10 B 11 Co 453 BO 3 BO 24 Co 383 Co 313 Co 513
			At 1%: BO 10 BO 11 Co 453 BO 3 BO 24 Co 383 Co 313 Co 513 Highly significant

non-protein (or "harmful") nitrogen, total ash content and ash in colloidal fraction (by incineration of colloidal precipitate obtained with rectified spirit and ether) and different ash constituents—Soluble SiO_2, $Fe_2O_3 + Al_2O_3$, CaO, MgO and P_2O_5. The analytical methods used have been referred to in a previous communication (1949), except in case of non-protein nitrogen which was estimated after Unverdorben and Spielmeyer (1939). Apart from the above chemical criteria, the colour readings of the juices (after dilution) were recorded in a Klett Sommerson Photo-electric colorimeter.

The two sets of gur samples resulting from the juices were examined with regard to general features and chemical and physical properties, *viz.*, sucrose (polarisation), glucose (reducing sugars), ash, nett rendement, acidity, colour and turbidity in standard solutions and pore space, as described in a previous communication (1949).

TABLE

Showing averages for properties of gur from different varieties with standard

[Treatments: (i) nil, (ii)

1 Variety / Characters	2 Co 313	3 Co 383	4 Co 453	5 Co 513	6 BO 3	7 BO 10	8 BO 11	9 BO 24
1 Sucrose% ..	84·43	75·50	77·78	80·15	78·15	78·35	80·63	80·44
2 Glucose% ..	2·55	3·89	2·78	3·20	4·17	3·40	4·64	3·12
3 Moisture% ..	4·19	4·65	4·67	4·52	4·55	4·48	4·48	4·45
4 Colour reading (N/4 solution)	91·25	196·50	255·00	89·38	202·50	196·13	196·00	196·13
5 Turbidity N/4 solution)	1·94	2·01	1·98	1·90	1·99	1·98	1·97	1·99
6 Milli *eq.* acid In 100 g	14·49	15·30	15·43	14·36	15·55	15·30	15·43	15·18
7 Pore space (cc. per 100 g.)	28·05	18·33	14·03	28·61	16·59	16·49	15·25	16·61
8 Ash% ..	2·40	2·90	3·37	2·33	2·82	2·83	2·81	2·85
9 Nett Rendement	73·43	61·47	63·19	68·80	64·11	65·03	66·17	67·30

II

errors per observation and critical differences at 5 and 1 per cent. levels

Castor seed extract]

10 S.E. per observation	11 C.D. at 5%	12 C.D. at 1%	Conclusions
±2·6048	2·58	3·44	At 5%: Co 313 BO 11 BO 24 Co 513 BO 10 BO 3 Co 453 Co 383 At 1%: Co 313 BO 11 BO 24 Co 513 BO 10 BO 3 Co 453 Co 383 Highly significant
±1·2716	1·28	..	At 5%: BO 11 BO 3 Co 383 BO 10 Co 513 BO 24 Co 453 Co 313 significant
±0·28	0·28	..	At 5%: Co 453 CO 383 BO 3 Co 513 BO 10 BO 11 BO 24 Co 313 significant
±50·93	51·21	68·38	At 5%: Co 453 BO 3 Co 383 BO 10 BO 24 BO 11 Co 313 Co 513 At 1%: Co 453 BO 3 Co 383 BO 10 BO 24 BO 11 Co 313 Co 513 Highly significant
±0·05	0·05	0·07	At 5%: Co 383 BO 3 BO 24 Co 453 BO 10 BO 11 Co 313 Co 513 At 1%: Co 383 BO 3 BO 24 Co 453 BO 10 BO 11 Co 313 Co 51 Highly sfgnificant
±0·48	0·4831	0·6451	At 5%: BO 3 BO 11 Co 453 Co 383 BO 10 BO 24 Co 313 Co 513 At 1%: BO 3 BO 11 Co 453 Co 383 BO 10 BO 24 Co 313 Co 513 Highly significant
±2·69	2·70	3·60	At 5%: Co 513 Co 313 Co 383 BO 24 BO 3 BO 10 Bo 11 Co 453 At 1%: Co 513 Co 313 Co 383 BO 24 BO 3 BO 10 BO 11 Co 453 Highly significant
±0·42	0·42	0·44	At 5%: Co 453 Co 383 BO 24 BO 10 BO 3 BO 11 Co 313 Co 513 At 1%: Co 453 Co 383 BO 24 BO 10 BO 3 BO 11 Co 313 Co 513 Highly significant
±3·32	3·32	4·44	At 5%: Co 313 Co 513 BO 24 BO 11 BO 10 BO 3 Co 453 Co 383 At 1%: Co 313 Co 513 BO 24 BO 11 BO 10 BO 3 Co 453 Co 383 Highly significant

TABLE III

Showing averages for gur/juice ratios in respect of sucrose, glucose and ash with standard errors and coefficients of variation

[Varieties: Cos 313, 383, 453, 513, BO's 2, 10, 11, 24. Treatments: (i) Nil, (ii) Castor seed extract]

No.	Ratio	Mean ± S.E.	Coefficient of variation
1	$\frac{\text{Sucrose per cent. in gur}}{\text{Sucrose per cent. in juice}}$	4·23±0·004378	0·104
2	$\frac{\text{Glucose per cent. in gur}}{\text{Glucose per cent. in juice}}$	9·92±0·0713	0·72
3	$\frac{\text{Ash per cent. in gur}}{\text{Ash per cent. in juice}}$	4·19±0 0168	0·40

TABLE IV

Showing averages in respect of the ratio $\frac{\text{Color per 100 Bx in juice}}{\text{Color per 100 Bx in gur}}$ *for different varieties and treatments with standard errors and coefficients of variation*

[Upper figures relate to treatment nil and lower figures to castor seed extract treatment]

No.	Variety	Mean±S.E.	Coefficient of variation
1	Co 313	2·69±0·0176	0·65
		5·13±0·0225	0·44
2	Co 383	1·98±0·0212	1·07
		4·12±0·4157	1·01
3	Co 453	1·74±0·0126	0·72
		3·70±0·0281	0·76
4	Co 513	2·61±0·0289	1·11
		4·79±0·1619	0·34
5	BO 2	2·06±0·0239	1·16
		4·31±0·0149	0·35
6	BO 10	2·25±0·0355	1·58
		4·64±0·8810	1·90
7	BO 11	2·26±0·0239	1·06
		4·63±0·9839	2·13
8	BO 24	1·95±0·0289	1·48
		4·02±0·1479	0·37

TABLE V

Showing averages in respect of time (in seconds) taken for temperature rise over different ranges with standard errors and coefficients of variation

[Varieties: Co's 313, 383, 453, 513, BO's 3, 10, 11, 24. Treatments: (i) Nil, (ii) Castor seed extract]

Range of Temperature (° C.)	Mean Time in Sec. ± S.E.	Coefficient of variation
1. 105– 6 ..	131·44±0·8634	0·66
2. 106– 7 ..	114·33±0·8870	0·78
3. 107– 8 ..	70·77±0·4639	0·66
4. 108– 9 ..	62·92±0·4886	0·78
5. 109–10 ..	60·67±0·3844	0·63
6. 110–11 ..	60·39±0·4349	0·72
7. 111–12 ..	61·77±0·4123	0·67
8. 112–13 ..	60·36±0·4192	0·69
9. 113–14 ..	59·55±0·4318	0·73
10. 114–15 ..	61·41±0·4346	0·71
11. 115–16 ..	59·72±0·3702	0·62
12. 116–17 ..	79·45±0·5151	0·65
13. 117–18 ..	115·08±0·7142	0·62
14. 105–18 (Whole range) ..	998·28±2·7737	0·28

TABLE VI

Showing correlation coefficients between different characters of juice and those of gur

[Varieties: Co's 313, 383, 453, 513, BO's 3, 10, 11, 24. Treatment: Nil]

1	2	3	4	5	6	7
Characters of juice / Characters of gur	Ash in colloidal fraction % Total ash in juice	Non-protein Nitrogen % Weight of juice	Protein Nitrogen % Weight of juice	Soluble SiO_2 % Weight of juice	Fe_2O_3 + Al_2O_3 % Weight of juice	CaO % Weight o juice
1 Sucrose %	−0·5054	−0·7056	+0·6145	−0·5020	−0·5927	−0·6485
	+0·0713	+0·4548*	−0·2788	−0·2197	−0·1989	+0·1763
2 Glucose %	+0·4283	+0·2921	−0·2516	+0·3889	+0·0521	+0·4311
	+0·0420	+0·0799	+0·4329*	+0·0581	+0·0467	+0·0608
3 Moisture %	+0·3023	+0·3222	−0·2029	+0·2033	+0·5079	+0·3878
	−0·3395	−0·0745	+0·1928	+0·1368	+0·3320	−0·0400
4 Colour reading (N/4 solution)	+0·9364†	+0·9606†	−0·9499†	+0·9570†	+0·9344†	+0·9582†
	−0·0702	−0·1695	−0·3701	+0·3598	+0·2217	−0·0583
Turbidity (N/4 solution)	+0·0705	+0·7134*	−0·7742*	+0·7332*	+0·6452	+0·6541
	+0·0332	+0·1456	+0·1446	+0·1660	−0·1884	+0·0363
6 Milli *eq.* acid in 100 g.	+0·9690†	+0·8602†	−0·8683†	+0·9471†	+0·8539†	+0·9366†
	+0·3706	−0·1660	−0·2539	+0·3666	−0·0276	−0·0233
7 Porespace (c.c. per 100 g.)	−0·9484†	−0·9364†	+0·9476†	−0·9769†	−0·8342*	−0·9461†
	+0·2987	−0·0821	−0·0401	−0·3048	−0·3348	+0·2603
8 Ash %	+0·8913†	+0·9023†	−0·8871†	+0·8999†	+0·9688†	+0·9079†
	+0·0224	+0·3907	−0·1385	+0·0922	−0·0564	+0·2658
9 Nett rendement.	−0·7002	−0·8230	+0·7472	−0·6968	−0·7368	−0·8108
	+0·0080	+0·2851	−0·4111*	+0·2230	+0·3147	+0·0893

N.B.—Upper figures are correlation coefficients *between* varieties and the lower figures those *within* varieties.

* denotes significance at 5% level, † denotes significance at 1% level,

TABLE VII

Showing correlation coefficients between different characters of juice and those of gur

[Varieties: Co's 313, 383, 453, 513, BO's 3, 10, 11, 24. Treatment: Castor seed extract]

Characters of gur \ Characters of juice	Ash in colloidal fraction % Total ash in juice	Non-protein Nitrogen % Weight of juice	Protein Nitrogen % Weight of juice	Soluble SiO_2 % Weight of juice	Fe_2O_3 + Al_2O_3 % Weight of juice	CaO % Weight of juice
1 Sucrose %	−0·4907	−0·7002	+0·6077	−0·4997	−0·5922	−0·6447
	+0·0627	+0·4517*	−0·2794	−0·2626	−0·2078	+0·1501
2 Glucose %	+0·4212	+0·2552	−0·2288	+0·3880	−0·00031	+0·4073
	+0·1539	+0·0881	+0·4269*	+0·0573	−0·0924	+0·0653
3 Moisture %	+0·5912	+0·6598	−0·5863	+0·5961	+0·5001	+0·6951
	−0·0680	−0·0888	+0·2309	−0·01028	−0·1273	+0·2699
4 Colour reading (N/4 solution)	+0·9461†	+0·9555†	−0·9472†	+0·9628†	+0·9244†	+0·9605†
	−0·2909	−0·4488*	−0·1586	+0·1051	+0·3084	−0·3926
5 Turbidity (N/4 solution)	+0·5280	+0·7528*	−0·8024*	+0·5629	+0·6819	+0·5696
	+0·1276	+0·1618	−0·1415	−0·5140†	−0·2038	+0·0675
6 Milli *eq.*, acid in 100 g.	+0·4986	+0·6505	−0·6354	+0·6092	+0·4033	+0·5803
	+0·3655	+0·0027	−0·2806	+0·2993	−0·04405	−0·2539
7 Porespace (c.c. per 100 g.)	−0·9693†	−0·9354†	+0·9531†	−0·9649†	−0·8448*	−0·9316†
	+0·3697	+0·1892	−0·1048	−0·2991	−0·4238*	+0·2207
8 Ash %	+0·8892†	+0·8923†	−0·8870†	+0·9009†	+0·9558†	+0·8987†
	+0·0416	+0·2883	+0·1230	+0·2772	+0·0404	+0·1683
9 Nett rendement.	−0·6959	−0·8127*	+0·7404*	−0·6999	−0·7057	−0·8045*
	−0·0237	+0·2695	−0·3911	−0·2588	−0·1285	+0·0636

N.B.—Upper figures are correlation coefficients *between* varieties and the lower figures those *within* varieties.

* denotes significance at 5% level. † denotes significance at 1% level.

In Table I, data pertaining to juices of different varieties are given, those for resulting gur samples being shown in Table II. Figures relevant to reproducibility of pan conditions will be found in Tables III to V and correlation coefficients between juice constituents and properties of gur in Tables VI and VII.

III. DISCUSSION

(*a*) *Varietal differences in juice constituents.*—A perusal of Table I, depicting average values for different juice constituents along with standard errors per observation and critical differences at 5 and 1 per cent. levels, brings out the following characteristic features in respect of the non-sugars in juices of different varieties:—

(1) Co. 313 and Co. 513 juices are associated with considerably lower levels of total colloidal matter, gums, pectin, ash and high phosphates, as compared to other varieties—Co. 453 showing the opposite behaviour in

all cases. The above fully confirms the authors' previous observations (*loc. cit.*).

(2) While differences in the total organic nitrogen contents are not significant, its distribution in the protein and non-protein (or " harmful ") forms is characteristically different. The preponderance of non-protein nitrogen (constituting about 70 per cent. of the total organic nitrogen) is remarkable in Co. 453 juices, in contrast to its low level (about 40 per cent.) in Cos. 313 and 513. The actual amounts of non-protein nitrogen in juice also follow a similar pattern, being highest in Co. 453 and lowest in Cos. 313 and 513. Protein nitrogen contents (obtained by difference) show exactly the opposite behaviour.

(3) Composition of the colloidal fraction with regard to organic and inorganic components is not characteristically different in juices of different varieties, in as much as the ash contents of this fraction exhibit no significant varietal variations.

(4) Distribution of the mineral matter in colloidal and molecular solutions is characteristically different, the highest proportions present in colloidal condition being found in Co. 453 juice in contrast to the lowest values exhibited by Cos. 313 and 513.

(5) Significant differences are manifested by several individual inorganic constituents (soluble SiO_2, $Fe_2O_3 + Al_2O_3$ and CaO), each of these being present in the highest amounts in Co. 453 juice. Cos. 313 and 513 show an exactly opposite behaviour. MgO contents are not significantly different.

(6) The function " Saline coefficient " (sucrose per unit ash) is highest for juices of Cos. 313 and 513, the least value being observed in Co. 453.

(7) Cos. 313 and 513 exhibit the lowest values for intensity of colour in juice, Co 453 (among others) giving the darkest juice.

In most respects, the varieties Co. 383, B.O. 3, B.O. 10, B.O. 11 and B.O. 24 occupy intermediate positions between the two extremes represented by Cos. 313 and 513 on the one hand and Co. 453 on the other.

It has to be remarked in this context that Ramanaya and Narasingha Rao (*loc. cit.*), in a comparative study of Co. 313 and Co. 419 juices, found relatively high values of silica, iron and aluminium oxide and magnesia (calculated on ash basis) and low values for CaO/MgO ratio and phosphate content in the former, as compared to the latter variety. While this might appear, at first sight, to be contradictory to the present observations, a comparison of the actual figures for Co. 313 (as shown below) would clearly

indicate that the present results are not entirely at variance with those of Ramanaya and Narasingha Rao:

	Ramanaya & Narasingha Rao		Present Authors
	Co. 419	Co. 313	Co. 313 (average)
SiO_2 per cent. ash ..	1·17	1·58	1·40
$Fe_2O_3+Al_2O_3$ per cent. ash ..	4·79	13·18	9·82
MgO per cent. ash ..	0·289	2·63	4·38
P_2O_5 per cent. ..	10·14	4·56	5·26
CaO/MgO ..	5·04	1·12	1·08

It is, however, clear that the criteria attributed to Co. 313 in the present investigations are fulfilled in a considerably greater measure by Co. 419. A study of the latter variety has not been possible as it was not one of the varieties under cultivation in Bihar.

Although preliminary work reported by Parashar (*loc. cit.*) would appear to indicate that Co. 313 is a high colloids variety (in contrast to the present observations), showing juice colloids of the order of 50 per cent. higher than in Co. 213 and some other varieties, detailed observations by the same worker, however, did not confirm the above but suggested that colloid content in raw juice was not a characteristic varietal attribute in Co. 313, being influenced largely by soil and climatic factors. There appears to be, therefore, no real divergence between the present results and the above observations with regard to the classification of Co. 313 as a high or low colloids variety and since a detailed study of colloid contents under the same set of soil and climatic conditions does not appear to have been conducted by the earlier worker (as in the present studies), a satisfactory comparison between the two sets of results cannot be effected.

(*b*) *Varietal differences in properties of gur.*—Average values for different properties of the gur samples from different varieties have been shown in Table II, along with standard errors per observation and critical differences at 5 and 1 per cent. levels. On an over-all estimate of the differences, in respect of various properties, it would appear that:—

(1) Cos. 313 and 513 are associated with the lightest colour, least mineral contents and insoluble impurities (as indicated by turbidity values), highest nett rendements, lowest acidities and most open texture (as apparent from

pore spaces). The products of these varieties are therefore of the highest quality.

(2) All other varieties are much below the level of Cos. 313 and 513 in all these respects and are therefore of a considerably lower order of quality. From the standpoint of several important criteria (particularly colour), Co. 453 may be considered as the most inferior in quality.

(*c*) *Evidence of reproducibility of pan conditions.*—Before attempting any correlation between the non-sugar ingredients in juices and the properties of their end products, it is necessary to examine the extent of reproducibility in pan conditions achieved in the experiments. If it can be shown that these conditions were controlled within satisfactory limits and were uniform all through the experiments, differences in the properties of gur (under a particular juice treatment) can be attributed entirely to inherent differences in the juices. Evidence of reproducibility has been adduced below in respect of: (i) ratios between sucrose, glucose and ash per cent. in gur and the corresponding values for juice, (ii) ratios between colour intensity per 100 brix in juice and the same in gur and (iii) uniformity in temperature gradients in the region of rapid rise (105–18° C.).

Table III, showing average values for gur/juice ratios in respect of sucrose, glucose and ash per cents along with standard errors and coefficients of variation, will reveal remarkable consistency in each case in as much as the coefficients of variation are considerably below the stringent 1 per cent. standard. The consistency with regard to sucrose ratio indicates that in all pans boiled, the changes affecting sucrose in juice (inversion, caramelisation, etc.) have taken a practically identical course. Similarly, the nett effect with regard to glucose (sucrose inversion, glucose destruction, etc.) may also be regarded as uniform. The same holds good for the changes involving mineral constituents (in clarification).

The figures pertaining to ratios between colour intensity per 100 brix in juice and gur have been incorporated in Table IV. This criterion will be found to vary with variety and juice treatment. For a particular variety under a given treatment, the coefficient of variation is almost always under the stringent 1 per cent. level. It may therefore be inferred that under these conditions the ratio is consistent, which indicates reproducibility with regard to the over-all effect of the processes affecting colour (removal of colloidal matter, caramelisation, reactions involving glucose, etc.) and although subject to the limitation that standard errors in this case were calculated on the basis of only four available observations, it will not be out of place

to advance this as additional evidence in support of what has been deduced from other considerations (sucrose, glucose and ash ratios).

Data on temperature gradients over the zone of rapid rise (comprising rab and gur making stages) will be found in Table V. It will be clear that the total time as well as the time periods for each degree rise in temperature over this range (105 to 118° C.) are highly consistent, with coefficients of variation always lying under 1 per cent. The course of boiling may thus be regarded to have been practically identical in all cases. In view of all the above observations and the fact that each pan was struck at exactly the same temperature (118° C.), it may be concluded that the experimental conditions were satisfactorily uniform and reproducible.

(*d*) *Correlation of juice criteria with properties of end-product.*—The correlation coefficients between (i) ash in colloidal fraction per cent. total ash, (ii) non-protein nitrogen, (iii) protein nitrogen, (iv) soluble SiO_2, (v) $Fe_2O_3 + Al_2O_3$ and (vi) CaO per cents in juice on the one hand and different properties of resultant gur (corresponding to no treatment) on the other have been depicted in Table VI. These reveal the following correlations existing *between* varieties:—

(1) Ash in colloidal fraction per cent. total ash in juice is correlated positively with colour, acidity and ash in gur and negatively with pore space which means that higher percentages of ash in the colloidal fraction give rise to products of dark colour, higher acid and mineral contents and less open texture. In other words, higher values of this criterion are detrimental to clarifying properties of juice and quality of product.

(2) Non-protein nitrogen in juice, apart from showing exactly the same correlations as above, is also positively correlated with turbidity of gur solutions which means that high values for this ingredient induce greater amounts of insoluble impurities in gur (besides increasing colour, acidity, ash and promoting a closed texture) and are therefore unfavourable.

(3) The correlations of protein nitrogen in juice with properties of gur are in all respects exactly opposite to those exhibited by non-protein nitrogen. High protein nitrogen content in juice is therefore a desirable criterion.

(4) Soluble SiO_2, $Fe_2O_3 + Al_2O_3$ and CaO contents in juice are correlated with properties of gur in the same manner as non-protein nitrogen. Low levels in respect of all these inorganic constituents therefore promote superior clarifying properties in juice and quality of end-product. The correlation coefficients pertaining to castor seed extract treatment in juice (set out in Table VII) lead to much the same conclusions. Coming to

correlations *within varieties*, it will be seen that coefficients here are generally much smaller than and sometimes of opposite sign to those *between* varieties (although not significant in both cases except in one erratic instance under castor seed extract treatment). Apparently variations in the different juice characters do not influence the properties of gur to any great extent *within a particular variety*, as do variations *between varieties*.

The following varietal juice characteristics (*i.e.*, as observed between varieties) conducive to superior clarifying properties and high quality of end product are, therefore, formulated:—

(1) Low non-protein nitrogen and high protein nitrogen, the former constituting not more than about 40 per cent. of the total organic nitrogen content.

(2) Distribution of the total mineral content in such a manner that relatively smaller proportions (not exceeding 6 per cent.) occur in the colloidal fraction.

(3) Low level of soluble SiO_2, $Fe_2O_3 + Al_2O_3$ and CaO (not exceeding about 6, 60 and 30 mg. per cent. weight of juice respectively).

IV. Summary

(1) In view of the great technological importance of non-sugar constituents of juices in vacuum pan practice, an examination of their bearing on clarifying properties in the relatively inefficient open pan process was felt to be necessary in order to arrive at the optimum criteria required of varieties for the open pan industry. The work represents an attempt undertaken with the above end in view.

(2) Certain relationships previously observed in this connection are confirmed.

(3) Examination of some new aspects lead to the formulation of several other characteristics associated with superior clarifying properties and high quality of end product, *viz.*—

(i) Low non-protein and high protein nitrogen contents.

(ii) Distribution of the total mineral content in such a manner that relatively smaller proportions occur in the colloidal fraction.

(iii) Low levels of soluble SiO_2, $Fe_2O_3 + Al_2O_3$ and CaO in juice.

V. Acknowledgment

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Central Sugarcane Committee, to whom grateful thanks are due. The assistance rendered by Mr. K. S. Bandyopadhyaya in statistically analysing data presented here is also acknowledged.

VI. References

1. Alvarez .. .. .. *Revista Ind. Agricola Tucuman*, 1938, **27**, 111.
2. Beater .. .. .. *Proc. 11th Ann. Congr. S. African Sugar Tech. Assoc.*, 1937, **82**.
3. Birkett .. .. .. *Trop. Agri.*, 1940, **17** (12), 233.
4. Browne and Zerban .. *Sugar Analysis* (*New York*), 1941, p. 1086.
5. Caminha .. .. .. *F. Brazil Azuc*, 1935, **4**, 335.
6. David, Gomez and Boon .. *Internat. Sug. Jour.*, 1938, **40**, 105.
7. Davis, Duncan and Yearwood *Ibid.*, 1936, **38**, 299.
8. Davis .. .. .. *Ibid.*, 1937, **39**, 67.
9. ——— .. .. .. *Ibid.*, 1939, **41**, 463.
10. ——— .. .. .. *Trop. Agri.*, 1940, **17**, No. 3.
11. ——— .. .. .. *Proc. 6th Congr. Internat. Soc. Sug. Tech. Louisana*, 1939.
12. Deerr .. .. .. *Cane Sugar* (*London*), 1921, p. 16.
13. Fleshman .. .. *Rept. Assoc. Hawaiian Sug. Tech.*, 1936, 121.
14. Guillaume .. .. *Proc. Assoc. Tech. Azuc. Cuba*, 1939, **9**, 65.
15. Gundu Rao and Kripashankar *Proc. Ind. Sug. Tech. Assoc.*, 1939, 8th Conv.
16. Hadfield .. .. .. *Hawaiian Planters' Record*, 1934, **38**, 126.
17. Hornby and Kasjanov .. *Zeit. Zuckerind. Czechoslov*, 1939, **63**, 187.
18. Kortscher .. .. *Hawaiian Planters' Record*, 1939, **43**, No. 1 (October).
19. Khanna and Prasad .. *Indian Farming*, 1947 (August), **382**.
20. ——— and Chacravarti .. *Curr. Sci.*, 1949, **18**, 19.
21. ——————— .. .. *Proc. Ind. Acad. Sci.* 1949, **30B**, 99.
22. ——————— .. .. *Ind. J. Agri. Sci.*, 1949, **19** (In Press).
23. ——————— .. .. *Ibid.*, 1949, **19** (In Press).
24. McCleery .. .. *Rept. Exp. Sta. Hawaiian Sug. Planters' Assoc.*, 1933, **36**.
25. Muller .. .. .. *Internat. Sug. Jour.*, 1921, **23**, 579.
26. Ness .. .. .. *Ibid.*, 1941, **43**, 378.
27. Parashar .. .. .. *Proc. Ind. Sug. Tech. Assoc.*, 11*th Conv.*, 1942. (Also in *Internat. Sug. Jour.*, 1933, **46**, 17).
28. ——— Kripashankar and Lal *Proc. Ind. Sci. Congr. Discussions*, 1947.
29. Pocy .. .. .. *F. Cuba Agricola*, 1934, **1** (8), 11.
30. Ramanaya and Viswanath .. *Proc. Ind. Sug. Tech. Assoc. 4th Conv.*, 1935.
31. ——— .. .. .. *Ibid.*, 5*th Conv.*, 1936.
32. Ramanaya and Satyanarayana *Ibid.*, 7*th Conv.*, 1938.
33. ——— and Narasingha Rao *Ibid.*, 1942, 11*th Conv.* (Also in *Sugar*, 1943, **35**).
34. Soragato .. .. .. *Internat. Sug. Jour.*, 1936, **38**, 532.
35. Springer .. .. .. *Proc. Jamaica Assoc. Sug. Tech.*, 1937, **1** (2), 30.
36. Unverdorben and Spielmeyer *Zeit. Ver. Deut. Zuckerind.*, 1939, **89**, 616.

STUDIES ON HORMONES

I. Effect of Hormone Injections of the Spikes of Wheat on the Metabolic Activities of the Leaves

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Preface

The broad title of the paper "Studies on Hormones" under which it is proposed to publish a series of papers from this laboratory, signifies the use of substances both of plant origin as well as chemical substances which effect plant metabolism in very minute amounts. The present paper, first of the series, deals with the effect of the hormones of the spikes of wheat on the metabolic activities of the plant. The work was carried out at four different reproductive phases of the plant, namely, (*a*) young spikes prior to emerging out of the leaf-sheath, (*b*) pre-anthesis, (*c*) anthesis, (*d*) post, anthesis, *i.e.*, 15 days after pollination. The following metabolic studies of the leaves at these four stages were studied, namely (1) Respiration, (2) Total nitrogen, (3) Amino nitrogen, (4) Sugars.

Material and Method

Vijaya (Ranjan[3]) wheats were used throughout the experiments. These plants were grown in the fields of the Botanical Garden.

Respiratory studies were made by bubbling air, issuing from the respiratory chamber, into petten-kofer tubes previously filled with baryta. The respiratory chamber was in its turn kept in a Hearson's electric incubator maintained at a temperature of $\pm 35°$. The current of air was automatically switched, after every 3 hours, into the next petten-kofer tube by the help of Blackman's air current commutator. Each tube was then lifted from its frame, washed and titrated against standard HCl. The amount of CO_2 given out by the leaves was then calculated.

Hormone extraction

About 20 gm. of spikes, each lot of a particular age group as mentioned before, were brought from the experimental field and were kept in the freezing chamber of the frigidaire overnight. They were then crushed to a moderate fineness. Ether was then added equal to 20 times the weight of the crushed material. The material was allowed to remain in ether overnight. The

following day the material was filtered and the filtrate was distilled off under reduced pressure. When all the ether was thus removed, 150 c.c. of distilled water was added to the residue and shaken thoroughly. This solution contained the crude hormone.

Injection

Leaves approximately of the same age were brought from the field with their excised bases dipping in a beaker of water. The control set and the experimental sets were then separately weighed and then injected. The method of injection was to dip the leaves in distilled water or the hormone solution, as the case may be, kept in a tube. The air then was removed by connecting it with a hand worked Garyk Vacuum Pump. When most of the air was withdrawn the negative pressure was slowly released to allow the liquid to get injected in the inter-cellular spaces. It was noticed by the senior author[3] in Cambridge that about 13% of oxygen enters the injected leaves. Thus, though there is undoubted restriction in the flow of gases, there is a sufficient supply of oxygen to the cells to maintain them above the anærobic level.

Total Nitrogen Estimation

The estimations of total nitrogen of the leaves, at four different stages, were done by Kjeldahl's method. Amino-nitrogen was estimated by the help of Vanslykes apparatus—with minor modifications.

Sugars

Sugar estimation was done by Somogyi's method with minor modifications. The following sugars were estimated: Mono and Di-saccharides, and total sugars after hydrolysis.

Observations

Respiration.—The first respiratory studies were undertaken on the leaves of the first stage, which is graphically depicted in Fig. 1. The respiration starts at 30 mg. CO_2 and drops off to about 18 mg. in 24 hours. This is the usual type of floating respiration. Fig. 2 gives the respiratory rate of leaves, at the second stage, *i.e.*, the pre-anthesis stage of the spikes. The curve of the continuous line shows the respiratory rate of leaves injected with hormone while the dotted line depicts the control. The difference in the respiratory rate is noticeable only during the first 9 hours and that too is not very pronounced. On the other hand, the respiratory rate at the 3rd stage (see Fig. 3) shows a significant difference between the experimental and control sets. For, whereas in the experimental set the respiration

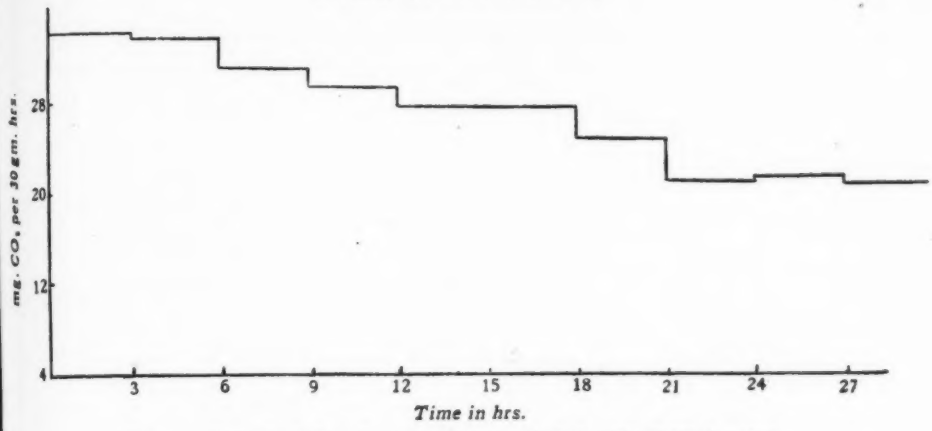

FIG. 1. Stage I : before flowering (leaves injected with distilled water)

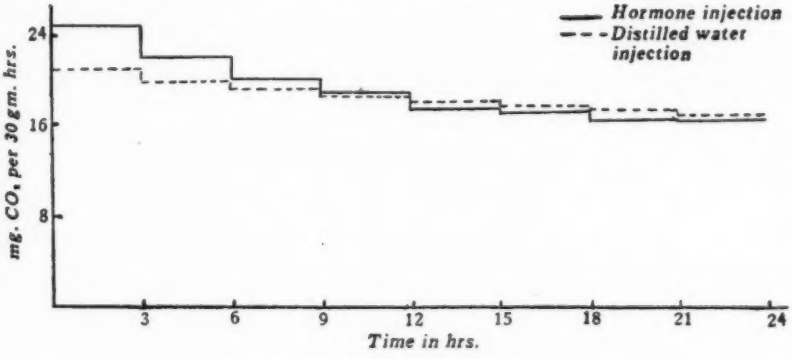

FIG. 2. Stage II : Pre-anthesis

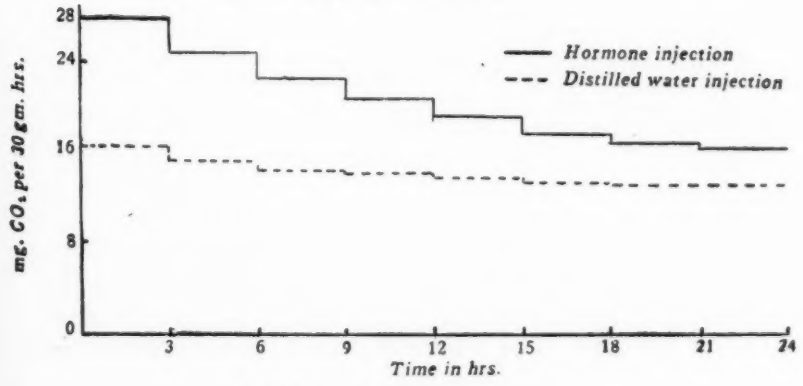

FIG. 3. Stage III : Anthesis

starts at 28 mg. CO_2 per 30 gr. hours in the control set it starts at 16. This difference, however, narrows down with time. There is a significant change in the shape of the respiratory curve at the 4th, *i.e.*, final stage. Unlike the

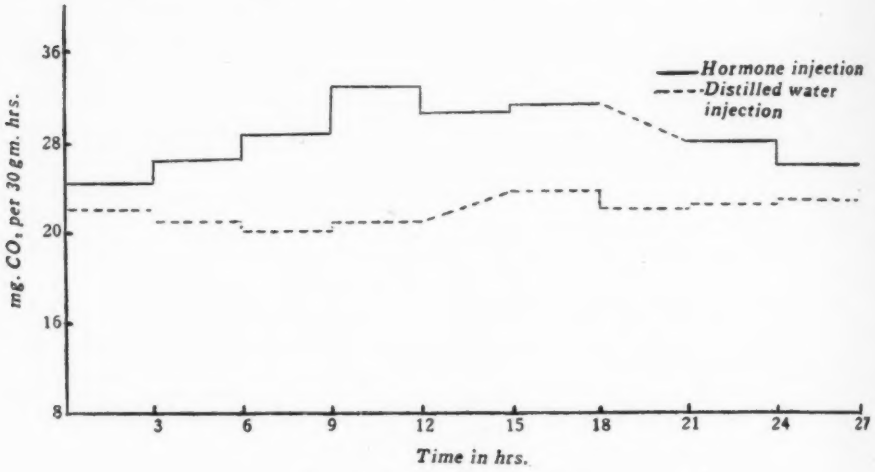

FIG. 4. Stage IV : Post anthesis

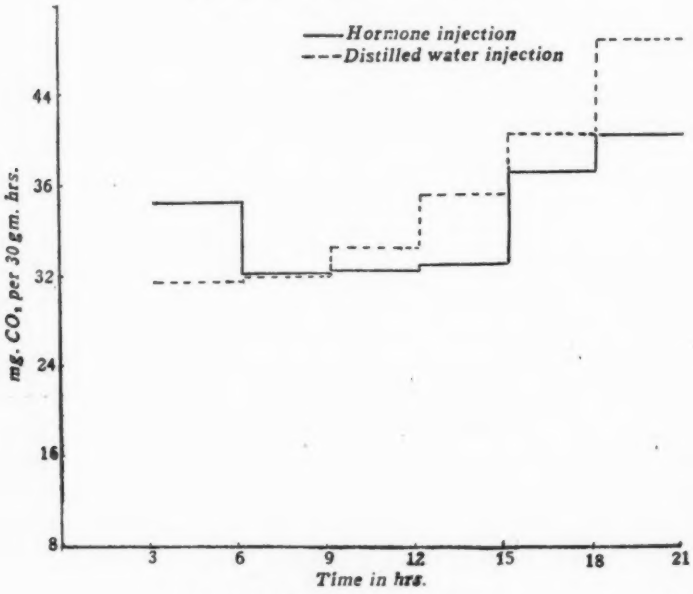

FIG. 5. Injection of leaf extract

previous case where the respiration of the experimental set started high, the respiration in this case started near about the level of the control set. However, the respiration gradually mounted up to reach a peak value at the end of the 12th hour. Thereafter it slowly dropped off (see Fig. 4). Fig. 5 shows the respiratory rate of leaves injected with no hormones from the spike but with an extract of the crushed leaves. The extraction was done in a manner similar to the extraction of the spike hormone. This was done only to find out whether any extract injected into the leaves effected its respiration. No noticeable effect could be recorded.

Total Nitrogen.—The study of total nitrogen reveals that during the first stage, that is before flowering the total nitrogen of the leaves is high as shown by Fig. 6. Thereafter there is a rapid fall in the two subsequent

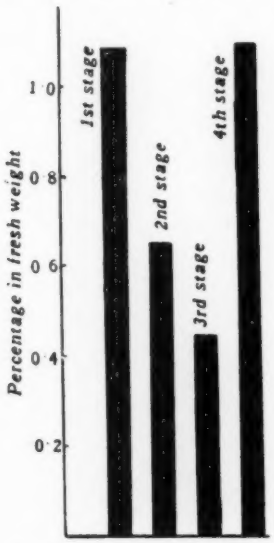

FIG. 6. Total nitrogen of four stages

stages while at the 4th and final stage it again rises. On the contrary the sugar fractions at different stages of the wheat plant reveals (Fig. 7) that whereas the hexoses increase in the third and fourth stages, there is a definite fall in the sucrose and the total content in the second and fourth stage. While this fall is not noticeable at the third stage.

The analysis of the sugars in relation to hormone injection (Fig. 8) shows that, inversely, as the hexose increases 24 hours after injection, there is a fall of the sucrose and total sugars.

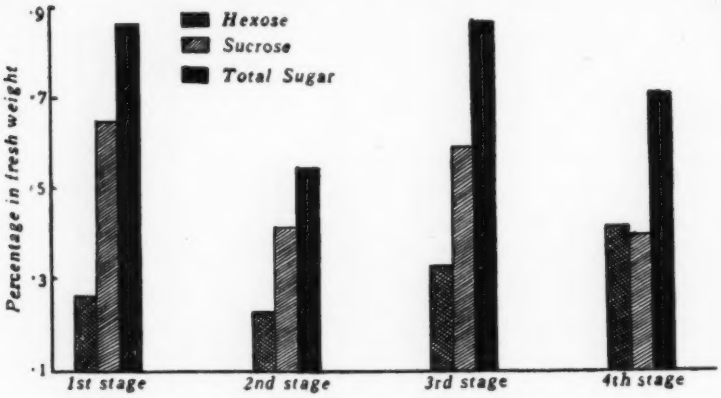

FIG. 7. Sugar fractions at four different stages

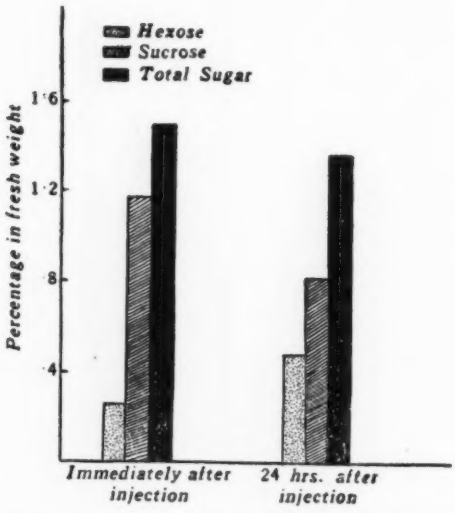

FIG. 8. Sugars in relation to Hormone Injection

A very noticeable feature of hormone injection is the variation in the amino N_2 as is shown in Fig. 9. The amino nitrogen before injection is slightly less than ·07 gm. per 100 gm. While 24 hours after injection of the hormone it increases to 0·11 gm.

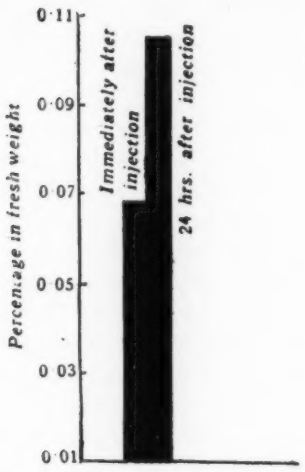

FIG. 9. Amino nitrogen in relation to Hormone Injection

DISCUSSION

According to Sande Bakhuysen[4] fertilization in wheat is a critical stage in the life of the plant. At gametic union growth hormones, produced in the inflorescence, no longer leave the flowers. He further goes to say that this is responsible for the catabolic impulse which depresses the rate of respiration and moisture content, hastens senescence and final death of the plant. But this somehow appears to be a contradictory statement. Because if the hormones of the spikes are unable to pass downwards to the leaves how is it that they control the catabolic impulses. Murneek[1] has also demonstrated that developing flowers and fruits have a marked effect on the metabolism of the plant and that they exert a certain amount of physiological control. The point, which so far is not very clear is, whether such control is evident only after reproduction has sufficiently progressed towards the point of completion, that is seed formation, or whether the effect is noticed at the earlier stages of reproduction. Again whether the effect is of one type at the earlier stages and a reverse type at the later stages of seed formation.

Soding[5] believes that it is the young inflorescence which controls the growth of the flower stem.

In a more recent work Wittwer[6] is of opinion that intervals of maximal increase in periods of greatest vegetative extension, accumulation of dry matter, etc., follows closely the beginning of syngamy in the flower bud and

embryo initiation in the young fruit. While agreeing generally with Wittwer's findings, in as much as that syngamy is an important stage, the injection of spike hormones in the leaves, leaves no room for doubt that the reactions are of a more complicated nature. On the one hand it is very clear from Figs. 2 and 3 that the production of the hormone is almost negligible upto the pre-anthesis stage; on the other at the mature stage or the stage when pollination has just taken place (Fig. 3), there is copious production of the hormone. It is not very clear that at this stage the abscission layer, at the base of the spike, is formed (anatomical studies will be necessary) for if it were so then neither water nor food material would travel from the leaves towards the spike. It should be remembered that the spikes are still green and the development of the young embryo is still to take place. Then how is it that the respiration rate of the leaves at this stage keeps at a low level. It is but logical to conclude that if no abscission layer is formed at this stage then the spike hormones should travel down. and by the increase of hydrolysis facility, augment the respiration rate. Yet this does not happen. It is possible that the young spikes at this stage are having intense meristematic activity and all the hormones that are produced are utilized to this end in the spike itself. Pal's[2] work at Rothamsted has shown that the green spikes also manufacture most of their carbohydrates. This will indirectly confirm the view that the hormones are so utilized in the spikes, and none become available for the leaves; and, therefore, the respiration keeps low (see Fig. 3). Further evidence is shown by the injection of the spike hormone to the leaf which causes rapid increase of the respiration rate, so that, if the hormone had travelled down, increased rate of respiration would have taken place. Another significant fact that emerges out is, that with the lapse of time, the effect of hormones wears out and at the end of 24 hours the respiration almost comes down to the level of the control set. This proves that to keep the respiration rate high, there must be a continuous production of the hormones. At the late mature stage, that is the fourth stage, the action of the hormones seems to be different. Here the effect is noticeable sometime after the injection, and, with time, there is a progressively increasing effect; the peak value being reached after a lapse of 9 hours. After this period the curve again falls off. This delayed action may be due to increased difficulty in the entry of the hormone extract, from the inter-cellular spaces into the paranchymatous cells within.

With regard to the formation of the abscission layer it will be more logical to assume that this layer is formed not at the 3rd but at the 4th stage, when the development of the embryo has gone far ahead and food and water are no longer required, at the region of the spike to the same extent. The

respiration rate of the leaves, at this stage, is the lowest. The total sugar is also less (see Fig. 7) though total N_2 is high.

That the hormones of the spikes have a profound effect in augmenting the metabolic rate is seen from Fig. 10. Here the continuous line represents the respiration rate during the first three hours of the 4 different stages, and the dotted line represents the rate at the end of 24 hours of these stages. Here at the pre-anthesis stage the respiration is lowest. It rises at anthesis period, which synchronises within maximum hormone production. Here too the total sugar is high (see Fig. 7). Due to senescence and the consequent fibrosis of the cells, the entry of hormones is delayed and only after 24 hours, indicated by dotted line in Fig. 10, its action is felt and the respiration rises.

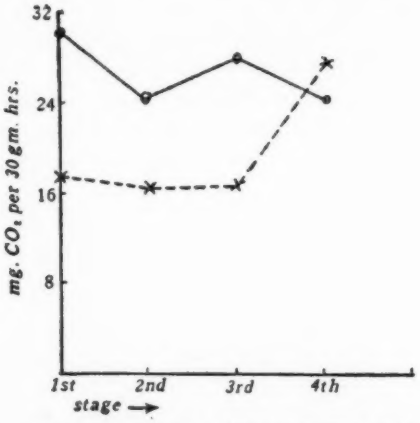

FIG. 10. Continuous line represents the initial respiration of four different stages Dotted line represents respiration rate of four stages at the end of 24 hours (Values taken from Figs. 1–4)

The effect of the injection of hormones upon the amine acid and sugar contents of the leaves also supports this. Fig. 8 shows that the amino-acids rapidly rises in 24 hours. We may, therefore, assume that the hormones increase hydrolysis facility of the proteins. This will explain the low values of total N_2 at the anthesis and post-anthesis stages; for these stages synchronise with the greatest hormone activity, which travelling downwards, towards the leaves, increase the hydrolysis of the proteins. The amino-acids so formed quickly get translocated to the growing spikes. Consequently the total N_2 of the leaves will fall. On the other hand, at the 4th stage, when the abscission layer has been formed translocation of the proteins are stopped, causing their accumulation in the leaves.

Further, these hormones not only increase hydrolysis facility of the proteins, but also of the carbohydrates (see Figs. 8 and 9). For, although the total sugars decrease, the hexoses increase. This is understandable in view of the increased respiratory activity, consequent upon hormone injection.

Summary

1. Hormones of the spikes of wheat extracted at four different stages, *viz.*, (*a*) Young spikes enclosed within the leaf-sheath, (*b*) Pre-anthesis (*c*) Anthesis, (*d*) Post-anthesis, *i.e.*, 15 days after pollination, were injected into the leaves, of ages synchronising with the 4 stages of the plant.

2. The injections of the extracts at the first two stages did not bring about any significant difference in their respiratory activity. But the extracts at the two subsequent stages, *viz.*, Anthesis and Post-anthesis had a marked effect; with this difference that the extract at stage 3 brought an immediate enhancement of the respiratory activity while at the 4th stage it reached its maximal at the end of 24 hours.

3. The hormone extract increases the hydrolysis facility of both the proteins and carbohydrates.

Bibliography

1. Murneek, A. E. and Wittwer, S. H. "Relation of Sexual Reproduction to Development of Horticultural Plants. General Effects of Flower and Fruit Production," *Proc. Am. Soc. Hort. Sci.*, **40**, 201–04.
2. Pal, N. L. .. .. Unpublished papers, London, 1946.
3. Ranjan, S. .. .. Unpublished thesis, 1925, Cambridge.
4. Sande-Bukhuysen, H. L. van de "Studies on Wheat Grown under Constant Conditions," Food Res. Inst., Stan University, 1937.
5. Soding, H. .. .. "Uber den Einfluse der jungen Infloreszenz das Wachstum ihres Schafters," *Jahrb of Wiss Bot.*, 1926, **65**, 611–85.
6. Wittwer, S. H. .. .. "Growth Hormone Production, during Sexual Reproduction of Higher Plants," *Research Bulletin*, 371, College of Agriculture, Universty of Missouri, 1943.

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OBSERVATIONS ON THE PROBABLE EFFECTS OF SALINITY ON THE SPAWNING, DEVELOPMENT AND SETTING OF THE INDIAN BACKWATER OYSTER, *OSTREA MADRASENSIS* PRESTON*

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1. Introduction

The salinity of sea-water, like its temperature, is one of the abiotic factors having a remarkable influence on the development of oysters as is well known from the work of Amemiya (1921, 1926 and 1928), Nelson (1921), Seno, Hori and Kusakabe (1926), Hopkins (1931), Gaarder (1932, 1933), and Gaarder and Bjerkan (1934) on different species of European, Japanese, Portuguese and American origin. Very little scientific information is available on the behaviour of the edible Indian backwater oyster in varying salinities. Hornell (1910 *a*) observed maximum sexual activity in this

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oyster on the east coast rivers and backwaters synchronizing with the heavy rains of October and November, and held that the chief stimulating factor in its spawning was not rise in temperature but a fall in salinity. Hornell (1922) observed also that on the Coromandal Coast there was a spawning maximum in March-April with cases of stray spawning individuals in between; in his view the spawning was induced not only by rise in temperature but also by fall in salinity. Sundar Raj (1930) remarked that "the salinity favoured by the breeding oysters, their eggs, and larvæ was found to range from 8·42‰ to 29·99‰ with a specific-gravity of 1·007 to 1·020." Panikkar and Aiyar (1939) observed motile sperms in the male oysters of Adyar River in two successive years, *e.g.* (November) 1934 and (October and November) 1935. Paul (1942) found them breeding in the Madras Harbour from April to October which constitute their reproductive period, and, on a minimum scale, during the non-reproductive period also. It will be seen that while Hornell's observations were based on backwater oysters, Paul's were confined to the same species found in the Madras Harbour. No salinity readings appear to have been recorded by either of them. Sunder Raj's statement quoted above lacks details, and the range of salinity stated to have been observed is too wide to be of any significance. The present studies are based on laboratory work and field observations both under marine and estuarine conditions over a continuous period of 18 months from March, 1948 to August, 1949. The diversity of breeding habits of the same species of oyster in two different localities due to direct or indirect effect of variations in salinity is of considerable scientific interest.

2. Material and Methods

Regular supplies of living *Ostrea madrasensis* Preston* were obtained for study at intervals of fifteen days both from the Madras harbour, which presents marine conditions, and the Adyar river-mouth with its adjoining backwaters, which has estuarine and brackish-water conditions. The gonads were examined macroscopically and also microscopically either by the direct observations of smears or by serial sections. Artificial fertilisation was tried to ascertain the fitness of the reproductive elements to undergo development. Records of the salinity and temperature of the waters of

* The Indian backwater oyster is very variable in form and different authors ascribed it to different species as follows: Hornell (1910 *a*)—*Ostrea cucullata* Born, Preston (1916)—*O. madrasensis* n. sp.; Annandale and Kemp (1916)—*O. virginica* Gmelin; Hornell (1922)—*O. virginiana* Gmelin; Moses (1928)—*O. virginiana* var. *madrasensis* Preston; Winckworth (1931)—*O. arakanensis* Sowerby; Awati and Rai (1931) and Paul (1942)—*O. madrasensis* Preston. In the present state of our knowledge, it would be best to retain the specific name *O. madrasensis* Preston, for the backwater oyster of Madras.

the sea and the estuary were maintained for the entire period of observations. During the spawning season which was ascertained by actual observations on spawning oysters both in the natural habitat and in the laboratory, the gonads of the female oysters were carefully cut open with a sterilised scalpel and the contents pipetted out into a petri-dish were mixed with similarly extracted male gonadic contents to bring about fertilisation by the dry method. For determining the favourable salinity medium for development, this mixture of eggs and sperms was placed in samples of water of varying grades of salinity in separate finger-bowls. The salinity of sea-water filtered several times through a plug of cotton-wool was estimated by titrating it with silver nitrate solution and checked up with standard solution of sodium chloride containing 32·34 gm. per litre. For all lower grades of salinity the sea-water was diluted with measured quantities of distilled water. For preparing grades of salinity higher than that of sea-water two methods were followed: (1) the addition of sodium chloride to fresh sea-water and (2) evaporation of sea-water in sunlight in enamel trays for raising its concentration. In both cases the exact salinity after the treatment was determined by titration with silver nitrate, and suitable grades were prepared by dilution of the medium with distilled water. The results obtained did not materially differ under experimental conditions. The first method was found suitable when a salinity of a desired grade had to be prepared fresh at the time of the experiment, whilst the second had the disadvantage of producing an adverse effect over development by the spoilage of the medium when kept for over a fortnight. The possibilities of the deleterious effect on developing eggs due to the proportions of the chemical constituents of sea-water being disturbed by the addition of sodium chloride were foreseen and the higher grades of salinity prepared in this way were, therefore, checked up with results obtained by the second method. Six drops of the mixture of the eggs and sperms were transferred simultaneously into separate finger-bowls holding mixtures of water of varying grades of salinity. The mixtures were kept cool by the bowls being placed in trays holding water one inch deep and being kept covered by thin moist cotton gauze to minimise effects of evaporation. The embryos and the larvæ developed in the various bowls were, from time to time, examined microscopically. Water in the finger-bowls was changed once in twenty-four hours with a siphon plugged with cotton-wool to prevent escape of the larvæ. Laboratory observations thus made on the developing eggs and larvæ in response to variations in salinity formed a convenient basis for the interpretation of the field observations. Regular plankton collections were obtained throughout the period of observations, from the sea in the vicinity of the harbour and also from the

Adyar river and backwaters to study the frequency of occurrence of the larvæ. Grown-up larvæ of oysters of recognisable shape were picked up under the binocular microscope with a finely pointed pipette and transferred to finger-bowls containing varying grades of salinity of water with a view to determine a medium suitable for the setting of spat. For want of suitable equipment and other facilities no elaborate field observations could be made; it was not possible, for instance, to collect plankton and water samples at different depths to ascertain the vertical distribution of the larvæ and correlate the same with variations in salinity in different strata, if any. All collections of plankton were made by the surface hauls and of the water samples in the closest proximity to the oyster beds. Cultch laid in the backwaters was invariably disturbed by fishermen who frequented the place, and the observations on setting of spat were therefore confined to frequent examination of all submerged objects and heaps of shells placed in the backwaters at suitable places for the larvæ to settle.

3. Salinity and Temperature of Waters of Adyar River and of Madras Harbour

The estuary of the Adyar river[1] with its adjoining backwaters and the Madras Harbour[2] to which the present investigations were confined present typical conditions of varied environments under which the oysters normally thrive. In Adyar the oysters occur on stones close to the mouth of the river, at the base of the southern embankment of the estuary extending upto a distance of about a mile by the side of the Theosophical Society, and also on the boulders of the Elphinstone Bridge and the smaller Bridge near the Adyar cemetery. This estuary, as in the case of nearly all coastal backwaters of the east coast, has no permanent communication with the sea. During the floods of the north-east monsoon the level in the waters rises and communication is established with the sea; and when there is a fall in the level of the waters thereafter, the breakers at the mouth of the river silt up sand to form a 'bar' cutting off the opening into the sea. Thus the opening and the closing of the bar is intermittent; hence fluctuations in salinity are very marked (*vide* Table IV and Fig. 1). In the harbour, the oysters thrive under marine conditions over the boulders, piers, rocks and derelict vessels. The waters of the harbour are always in open communication with the sea.

Salinity Variations in Adyar.—From 13th March 1948 to 6th July 1948 the salinity was steadily rising from 20·32 to 38·47‰ (*vide* Table IV and

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1, 2 Detailed descriptions of the Adyar estuary and the Madras Harbour are given by Panikkar and Aiyar (1937) and Paul (1942).

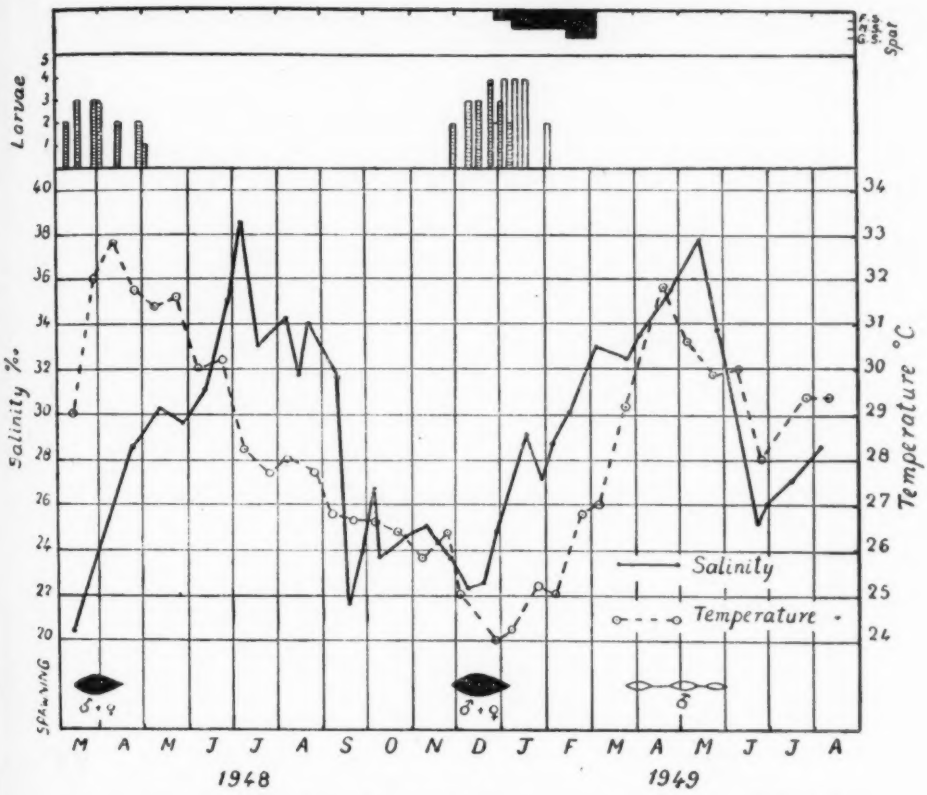

FIG. 1. Periods of spawning and frequency of occurrence of larvæ and spatfall of *Ostrea madrasensis* in relation to variations in salinity and temperature in Adyar estuary. Data presented in Table IV. (Explanation of lettering is as for Table IV: histograms with cross stripes indicate larvæ of young size with straight hinge stage, and those without cross stripes represent larvæ of setting stage.)

Fig. 1). There was a rapid rise in the temperature of the waters of the estuary owing to its shallowness and the consequent rapid evaporation. The loss of water due to evaporation could hardly be made good by the very meagre inflow of river water which also contributed to the bar being closed during this period. The local rainfall was poor.

On the 20th July 1948 the salinity dropped to 33·01‰ and varied thereafter upto 12th September 1948 between 34·43 and 31·74‰. During this period the temperature was falling resulting in lesser evaporation than before, and there was also more river water entering the estuary to reduce the salinity. The bar was still closed.

On the 26th September 1948 there was a sudden drop in salinity of the estuary due to heavy floods in the river. This happens almost every year before communication is established with the sea.

During October, November and December of 1948 the salinity continued to be low, except for a slight rise up to 26·8°/$_{oo}$ on 11th October 1948 due probably to the establishment of a communication with the sea for a short time which escaped attention.

From 29th December 1948 to the last week of January 1949 the salinity ranged between 24·89 and 29·01°/$_{oo}$ when the bar was open and the estuary communicated with the sea.

During February 1949 when the communication of the estuary with the sea was intermittent the salinity was 28·87°/$_{oo}$.

From 5th March 1949 to 14th May 1949 there was a rise in salinity from 33·00 to 37·94°/$_{oo}$ due to the closure of the bar and the prevailing high temperatures and the consequent rapid evaporation of the water as in the corresponding period of the year 1948.

The fall in salinity recorded in June 1949 was due to heavy local rainfall in the previous month.

The Salinity Variations of the Sea-water in the Vicinity of the Harbour.—For the most part of the year the salinity ranged between 32·00 and 35·00°/$_{oo}$, except for sharp variations due to rise in temperature in March and April 1948 and April 1949 up to 36·97°/$_{oo}$ and 37·25°/$_{oo}$ and 36·65°/$_{oo}$ respectively, and rapid fall ranging between 30·55 and 24·6°/$_{oo}$ from the second week of October 1948 to January 1949 due to influx of flood water from land after the monsoon rains.

Water Temperatures in Adyar.—The highest maximum temperature of the waters recorded from the oyster beds in Adyar was 32·8° C. in April 1948 and the lowest 24·2° C. in December 1948. In general high temperatures about 30 to 33° C. prevailed in April, May and June, and somewhat lower temperatures of 28° to 29° C. in July and August. During December and January the temperature readings were the lowest, ranging between 25 to 24° C., and by the end of February there was a tendency to rise again.

Water Temperatures of the Harbour.—The maximum temperature readings of 29° to 31° C. for the coastal waters of the sea in the vicinity of the Harbour were recorded during the periods April to June 1948 and May to August 1949. During the periods September-November and December-January the temperature came down to about 26° to 27° C. and 25° to 24° C. respectively. By the end of February it was shooting upto about 27° C.

In general, the maximum temperature during March to June in Adyar was a little higher than in the vicinity of the harbour which was to be expected in a relatively smaller and shallower area of the estuary which takes up heat more readily than the waters of the open sea.

4. Gonadic Condition and Spawning of the Backwater Oyster

The gonads are paired and very much alike in external appearance in both the sexes. They are creamy white, much branched with the ramifications of the tubular follicles spreading beneath the general epithelium and filling up the bulk of the visceral mass when the oyster is ripe. The follicles of the gonad of the two sides almost coalesce at the hinge, where they form a very thick mass, but are absent beneath the œsophagus and also in the region of the pericardium. The gonoduct of each side opens into a urinogenital cleft close to the adductor muscle. The ramifications of the gonadic tubules in a fully ripe female oyster are usually clearly seen through the epithelium whereas those of the ripe male are indistinct. In a partially spent female or male the part of the gonad close to the hinge is flabby with the accumulation of a certain amount of a watery fluid within, but farther down this region it is full of the whitish reproductive elements in the follicles. The amount of watery fluid varies depending upon the extent of spawning. The fully spent female oyster presents usually a more flabby appearance than its male counterpart, as the amount of the residual ova is always much less than the residual sperm in the spent gonads.

Gonadic Condition of Oysters from Adyar.—The condition of gonads of Adyar oysters exceeding two inches in length during the period of observations is given in Table I. From June to August both in 1948 and 1949 the proliferating activity of the gonads was observed as a preparation for the next spawning season. However, the differences in the condition of the gonads of March-April 1948 and of 1949 seem to be due to the salinity variations in the respective years. After heavy spawning and rapid resorption of the few residual reproductive elements, as it occurred in May 1948, it is difficult to distinguish the sex in a small number of oysters, and this phase may correspond to Loosanoff's (1942) 'indifferent stage' of *Ostrea virginica*, from which reversal of sex is possible. A few of the hermaphrodite phases noted in this species will be dealt with elsewhere. The occurrence of stray individuals with full gonads after the spawning period was not uncommon.

Gonadic Condition of Oysters of the Harbour.—The oysters of the Madras Harbour living under purely marine conditions do not seem to attain the

TABLE I

Changes in the gonadic condition and occurrence of spawning in relation to salinity and temperature variations in Adyar

Dates and months	Gonadic condition	Spawning	Salinity parts per thousand	Maximum temperature °C.
March to April, 1948	Full or partially spent or spent	Excellent spawning of both sexes	20—28	29—33
May, 1948	Spent	No spawning	30	31
June to 14th September, 1948	Recovering stage	No spawning	31—38	27—30
21st September to October, 1948	Full	Pre-spawning stage	21·7 to 26·8	26·4 to 26·8
November, December 1948 and 1st week of January, 1949	Full or partially spent	Excellent spawing of both sexes	22·39 to 25·08	24—25
Late January and February, 1949	Signs of recovery but no rapid proliferation	No spawning	29—30	24·8 to 26·8
March, April and May, 1949	Cytolysis and dissolution of eggs	Stray spawning of males only	32 to 37·9	27 to 31·8
June to August 1949	Stage of recovery	No spawning	27–30	28—30

proportions of those of Adyar living under the estuarine and backwater conditions either in the size of the animal or thickness of the gonad. The grown-up oysters in the Harbour were in a partially spawned condition throughout the year with relatively large number of developing oocytes in August and September.

Spawning in Oysters from Adyar.—Oysters from Adyar estuary placed in glass troughs in the laboratory spawned profusely on the 11th March 1948. At first a small male commenced emitting a steady stream of milt, which was followed by quick emission of eggs by two females of fair size; then more males and females spawned rendering the water in the trough cloudy. The salinity of the waters in Adyar was 20·32‰ and of the sea water 34·42‰. Spawning took place in sea-water diluted with tap-water so as to bring down its salinity to the level of the Adyar water. The temperature of the water in which spawning took place was 27°·3 C.

Natural spawning was observed on the oyster-beds when the salinity was 20·3‰ and the temperature 29°·0 C. at 12 Noon on the 13th March 1948.

It would appear that a large number of individuals in shallow waters near the southern embankment of the estuary had spawned simultaneously. Spawning in the laboratory continued at short intervals till the third week of April 1948. No further spawning was observed in the laboratory or in the natural habitat from the last week of April 1948 to third week of November 1948. In the last week of April 1948 when the spawning had just stopped the salinity of the estuarine water had risen to 28·59°/$_{oo}$. Again on the 27th and 30th November, 1st, 6th, 24th and 26th December 1948, and 4th and 6th January 1949 there was spawning of the oysters of both the sexes from this locality. In the spawning period of November to January spawning was observed in the natural habitat only on the 6th and 26th December, but on other days it took place under laboratory conditions. From the third week of November 1948 to the first week of January 1949 the salinity and temperature ranges were respectively 22·52–25·08°/$_{oo}$ and 24·0–26°·4 C.

Between the 6th January and the third week of March 1949 there was no spawning. On the 25th March, 1st April and 20th May 1949, when the water temperatures were high ranging from 29°·2 to 31°·8 C. only the males spawned. From the last week of March to the last week of May 1949, the salinity range was between 32·45 and 37·94°/$_{oo}$ which was considerably higher than it was during the previous period of effective spawning of both the sexes. Though the observations were discontinued by August 1949, grown-up larvæ were again seen in the Adyar estuary and backwaters in December 1949, which indicates spawning also in the November-December period of 1949.

From the field and laboratory observations on *O. madrasensis* it would appear that intense sexual activity occurs in the Adyar estuary during November-December, usually followed by a feeble one in March-April. All the salinity readings taken during spawning of both sexes fall within the optimum range, 22·00 to 26·00°/$_{oo}$ required for the development of the early veligers from fertilised eggs as corroborated by laboratory experiments. It is therefore probable that spawning in the backwaters takes place only when optimum salinity conditions prevail. The successful spawning of oysters in March and April 1948 and the failure of the same in the corresponding period of 1949 may be attributed to the favourable and unfavourable salinities prevailing respectively in the two years. It seems likely that the females and males behave differently under high salinities and temperatures, the former failing to spawn in the backwaters in increased salinities and the latter reacting positively to increased temperatures as they did in March to May 1949. It may also be observed that in March 1948 spawning oysters

continued to liberate spawn for a short time under laboratory conditions in pure sea-water of high salinity of 34·4‰ as well as in lower salinities. This seems to indicate that what constitutes the favourable condition for spawning is not the sudden change in salinity but the length of the period of stable salinity preceding spawning. Attempts at inducing the Adyar oysters to spawn in the non-reproductive period in samples of water of different grades of salinity met with no success.

Spawning of the Oysters of the Harbour.—Spontaneous spawning of the harbour oysters was not observed either in the natural habitat or under laboratory conditions, but induced spawning was, however, possible in April-May and October-November of 1948 and in March to May of 1949. Both sexes were induced to discharge their reproductive elements by the stimulus of the mere presence of ripe sperms and eggs in the water containing the oysters. The occurrence of oyster larvæ in the coastal plankton in all the months of the year is a positive evidence of more or less continuous spawning throughout the year.

5. Early Development of Oyster Eggs in Different Grades of Salinity

The experiments and observations of Amemiya (1921, 1926 and 1928) with fertilised eggs of oysters in various grades of salinity have shown that the optimum range of salinity for the development of shelled veligers varies with the species—25–29‰ in *Ostrea virginica*, 28–35‰ in *Gryphæa angulata*, 15–25‰ in *Ostrea gigas* and *Ostrea rivularis*, 28–38‰ in *Ostrea circumpicta* and 30–38‰ in *Ostrea spinosa*. Seno, Hori and Kusakabe (1926) and Clark (1935) made similar observations on *Ostrea gigas* and *Ostrea virginica* respectively. Awati and Rai (1931) found a density of sea-water of 1·020 or 1·021 to be most suitable in the early development of *O. cucullata*. Roughley (1933) observed free-swimming stages formed in the Australian, *O. commercialis* in 8, 9 and 10¾ hours in densities of 1·021, 1·015 and 1·011 respectively, the rate of development decreasing as the density was lowered. In a density of 0·005, most of the eggs remained unfertilised, but the few that passed through the segmentation stages did not develop beyond the morula stage. Cole (1939) found a salinity range of 30·5 and 32·5‰ suitable for the culture of *O. edulis* at Conway. The oviparous oysters are better than the larviparous ones for studying the effect of salinity as the eggs of the former can be more easily fertilised and developed normally in suitable media. As in larviparous *O. edulis* and *O. denselamellosa* artificial fertilisation was not successful, the embryos taken from the parent oysters were subjected to grades of salinity by Amemiya (1926 and 1928). The

effects of very high or very low salinities appear to be either cessation of development or retarded or defective segmentation. In the latter case the resulting blastomeres tend to clump together, like small colonies of yeast cells, developing into abnormal embryos and larvæ, or get detached to grow into incomplete larvæ.

In the experimental data detailed in Table II, altogether thirty grades of salinity media were used with laboratory temperatures varying between 26°·0 C. and 28°·5 C. Development was at its best in grade 17 of salinity 26·00°/$_{oo}$. The average time taken by the eggs to reach the respective stages after fertilisation in different grades of salinities is shown in the table.

It may be seen from Table II that in grades 1 to 3 of salinities ranging from 50·10 to 48·00°/$_{oo}$ and in grade 30 of salinity 4·33°/$_{oo}$ no development was observed. In very high salinities the eggs were very much shrunk and in very low salinities they were a little swollen. In grades 4 to 29 of salinities ranging from 46·00 to 6·5°/$_{oo}$ development was initiated by the extrusion of polar bodies and segmentation, and they are grouped under the following five ranges for convenience of description.

Range I of Too High Salinity.—In grades 4 to 9 of salinity ranges between 46·00 to 37·01°/$_{oo}$ development was defective, abnormal and incomplete. In the higher grades of this range the majority of eggs remained unchanged, and in the lower grades segmentation improved. In salinities from 41·00 to 37·00°/$_{oo}$ shell-formation was commenced after the trochophore stage but was not completed. There were only varying numbers of abnormal larvæ but no shelled veligers.

Range II of High Salinity.—In grades 10 to 16, of salinity ranges between 36·00 to 28·04°/$_{oo}$ development progressed satisfactorily in varying numbers of the eggs upto the shelled veliger stage. Segmentation was good and quite normal in most cases. With the fall in salinity the number of abnormal larvæ gradually decreased while the shelled veligers increased correspondingly. In the last two grades of this range, development was good except that some did not develop into shelled veligers even after 30 hours.

It may be observed here that, judging from the occurrence of the planktonic larvæ, the oyster seems to breed under marine conditions of the harbour for the most part of the year within the salinity limits of Range II. Fall in salinity of the coastal waters in October to about the same as in Range III, was followed by an increase in the numbers of larvæ.

Range III of Optimum Salinity.—In grades 17 to 20 of salinities from 26·00 to 21·83°/$_{oo}$ development was excellent and almost all the eggs divided

TABLE II

Showing Effect of Different Grades of Salinity on the Developing Eggs of the Oyster

Range	Grade No.	Salinity °/₀₀	Extrusion of polar bodies	Nature of segmentation	Duration of formation of Blastula	Duration of early free swimming stage	Duration of formation of Trochophore	Duration of Formation of shell	Duration of Formation of early Veligers	Abnormal free swimming larvæ
No development	1	50·10	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	2	49·72	do	do	do	do	do	do	do	do
	3	48·00	do	do	do	do	do	do	do	do
Range I Salinity—too high	4	46·00	30 min.	Defective	do	do	do	do	do	do
	5	45·00	do	do	do	do	do	do	do	do
	6	43·10	do	do	do	do	do	do	do	Large Nos.
	7	41·40	15 min.	do	6 hrs.	8–9 hrs.	20 hrs. (fair)	Not completed	do	Numerous
	8	39·00	do	do	do	do	do	do	do	do
	9	37·01	do	do	do	do	do (good)	do	do	do
Range II Salinity—high	10	36·00	do	Good	3 hrs.	6 hrs.	Large Nos.	24 hrs.	30 hrs. (very few)	Large Nos.
	11	35·00	do	do	do	do	do	do	do	do
	12	33·14	do	do	2½ hrs.	do	do	do	30 hrs. (a few)	do
	13	33·09	do	do	do	do	do	do	do	do
	14	31·34	do	do	do	do	do	do	do (fair)	Good Nos.
	15	29·69	do	do	2 hrs.	4–6 hrs.	18–20 hrs.	20–24 hrs.	28 hrs. (large)	Few
	16	28·04	do	do	do	do	do	do	do	Nil
Range III Salinity—optimum	17	26·00	1st 5–10 min. 2nd 15 min.	do	1¾–2½ hrs.	3½ hrs.	12–14 hrs.	17–18 hrs.	20–22 hrs. (numerous)	Nil
	18	24·86	do	do	do	do	do	24 hrs.	26–28 hrs. (numerous)	do
	19	23·83	do	do	do	do	do	do	do	do
	20	21·83	do	do	do	do	do	do	30 hrs.	do
Range IV Salinity—low	21	19·5	do	Defective	3 hrs.	5–6 hrs.	14–18 hrs.	24–30 hrs.	34 hrs. (few)	Very few
	22	17·33	do	do	do	do	do	do	do	do
	23	16·00	do	do	do	do	do	do	do	do
Range V Salinity—too low	24	15·10	do	do	do	do	do	Not completed	Nil	Large Nos.
	25	13·00	30 min.	do	3–3½ hrs.	do	20 hrs.	Nil	do	do
	26	12·43	do	do	do	do	do	do	do	do
	27	10·83	do	do	do	do	do	do	do	Numerous
	28	8·66	do	do	do	24 hrs.	60 hrs. (a few)	do	do	do
	29	6·5	do	do	Nil	Nil	Nil	do	do	Nil
No development	30	4·33	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

normally. There were no abnormal or partial larvæ. Almost all the eggs developed to the shelled veliger stage.

The salinity limits under which successful spawning of both sexes of the oysters took place in Adyar backwaters fall approximately within this range.

Range IV of Low Salinity.—In grades 21 to 23 of salinities from 19·5 to 16‰ development was retarded, defective and abnormal in varying number of eggs. Mortality was great after the free-swimming stage was reached. Some developed into the shelled veliger stage, thus completing the early development.

Range V of Too Low Salinity.—In grades 24 to 29 of salinities from 15·0 to 6·5‰ development was very much retarded, defective, abnormal and incomplete. After the trochophore stage shell formation commenced in the higher salinity grades within this range but was not completed. In most cases development did not proceed beyond the trochophore stage. There were numerous abnormal larvæ, but no veligers.

6. Frequency of Occurrence of Oyster Larvæ in Plankton Hauls

The relative abundance of the oyster larvæ in the plankton was measured by a rough method, which is a slight modification of procedure described by Hopkins (1931). An arbitrary standard was adapted in which numbers, *viz.*, 0, 1, 2, 3, 4 and 5 indicate none, very few, few, fair large and abundant numbers of larvæ respectively in each collection. As younger larvæ in the straight-hinge stage are difficult to be distinguished from those of other bivalves, the relative abundance of only larvæ of setting size was estimated. Where appearance of younger larvæ coincided with the spent condition of the gonad their occurrence was recorded with 1* in Table IV. Every plankton collection consisted of four horizontal hauls, each of fifteen minutes duration, the net being towed from a catammaran.

Oyster Larvæ in the Adyar Backwaters.—Following spawning early veliger larvæ occurred in gradually diminishing numbers in the backwaters and the estuary from the middle of March to the first week of May 1948. The average water temperature was 32°·8 C. rising upto a maximum of 33°·2 C. on 9th April 1948. This high temperature resulted in heavy mortality of the larvæ and the absence of spat. The salinity for most part of this period ranged between 20·32 and 28·59‰ which is almost the same as the optimum requirement for early development under controlled experiments. No larvæ were observed from the second week of May to the third

week of November 1948. From 26th November 1948 to 6th January 1949 there was spawning followed by the appearance of larvæ in gradually increasing numbers, the peak of occurrence of setting larvæ being in the first three weeks of January 1949 during which the salinity and temperature were 29·01‰ and 24°·2 to 25°·2 C. respectively. This was followed by a good spatfall. Between February 1949 and August 1949, when the observations were discontinued, no larvæ were present in the backwaters. In December 1949, however, grown up larvæ were observed in the same locality when the salinity was 21·90 and 22·50‰. Though spawning takes place in the backwaters under the optimum salinity of 22·0 to 26·0‰, the larvæ thrive well in slightly higher ranges as well, the bar opening usually at about this time letting in sea-water into the backwaters which raises the salinity.

Oyster Larvæ from the Sea in the Vicinity of the Harbour.—Varying numbers of larvæ of setting size were obtained from the plankton collections over a period of 18 months with peak occurrences in June-July 1948, November 1948 to January 1949, and May to July 1949. It may be seen from Table IV that the May–July period both in 1948 and 1949 was itself a period of very high temperatures or was preceded by a period of very high temperatures, whereas the November–January period was preceded by one of moderate temperatures and low salinity. Thus it follows that under marine conditions the backwater oyster remains sexually active throughout with intensive spawning periods which are followed by high temperatures, or low salinities suitable for early development.

7. Field and Laboratory Observations on Relation Between Salinity and Setting of Oyster Spat

Spat-Setting in the Backwaters.—The field experiments on the setting of spat did not prove a success, owing to the frequent disturbance caused in the locality by fishermen and clam gatherers. These experiments were made with (i) suspended dried shell-cultch, (ii) a small wooden rack with removable glass slides and (iii) heaped dead shells in shallow water, which could be examined, cleaned or replaced from time to time. Besides these experiments with cultch careful examination was made from time to time of all likely cultch in the locality such as the cement river embankment, the boulders of the Elphinstone Bridge across the Adyar river, and submerged stones or other hard objects on which spat may be expected to settle.

In March–April 1948 the absence of spat may have been a result of larval mortality due to high temperatures, unless a few that had settled in the cooler and deeper waters covering the boulders in the middle of the river

had excaped observation. Nor did the shells of oysters and other molluscs obtained from the river bottom by diving reveal any trace of spatfall.

The November–December spawning of 1948 was followed by the occurrence of larvæ till about the end of January 1949. On 29th December 1948, when the salinity had gone up to 25·49°/$_{oo}$ (*vide* Table IV) in the backwaters of Adyar owing to the bar having opened two days previously, grown-up larvæ were obtained in the plankton and it was possible to rear them to the spat stage in the laboratory in sea-water of salinity 25·98°/$_{oo}$. Spatfall continued in the estuary and the backwaters throughout January and the first week of February 1949 when the salinity varied between 27·32°/$_{oo}$ and 29·01°/$_{oo}$. In the extensive backwaters of Ennore, eleven miles north of Madras, widespread spatfall was observed on 21st December 1949, where the salinities were 31·09°/$_{oo}$ and 30·21°/$_{oo}$ in the shallow and deeper waters respectively. The bar was open and the backwater was in communication with the sea. In the same week in the Adyar backwaters where the salinity range was 21·92–22·52°/$_{oo}$ a fair number of larvæ was present but without spatfall. The observations made above seem to show that the optimum salinity for the spatfall is higher than that for spawning and early development, which under eontrolled experiments was found to be 22·00 to 26·00°/$_{oo}$. Spatfall was the best in both the backwaters when the salinities were as high as 29·00 to 31·09°/$_{oo}$. The absence of spat in Adyar in December 1949 may have been a result of the then prevailing low salinity owing to the bar remaining closed. To ascertain a suitable grade of salinity for setting, planktonic larvæ of setting size were placed in bowls of water of different grades of salinity.

Spat Setting in and around the Madras Harbour.—Frequent searches of the boulders, piers and other submerged objects of the locality for spat revealed that setting was at its best from (i) May to July 1948, (ii) December 1948 to January 1949 and (iii) May to August 1949, though larvæ continued to occur all the year round during the period of observations. Intensive spatfall was observed only when there was an abundance of larvæ in the sea.

Setting of Oyster Larvæ under Experimental Conditions.—Larvæ of setting size from the plankton of the inshore waters were carefully picked up under the binocular microscope with finely drawn glass pipettes and removed into finger-bowls containing different grades of salinity of sea-water, dilutions being made, with distilled water. Twenty larvæ were taken into each finger-bowl. Usually over 50°/$_{o}$ of them settled on the sides of the glass bowl in about 24 hours if the medium was of a suitable grade. Most of the setting took place during the night. If the setting of larvæ was

delayed, fresh sea-water of the same dilution was added after the old water had been carefully siphoned out. The experiment was repeated with different grades of salinity prepared out of estuarine water obtained from Adyar when the bar was closed.

During the periods when good spat were obtained in the vicinity of the harbour (*vide* Table IV), laboratory experiments on setting also met with success. The months and salinities of sea-water in which excellent setting took place are shown below:—

Salinity of sea water	33·01°/₀₀	28·39°/₀₀ & 29·07°/₀₀	33·21°/₀₀ 33·39°/₀₀	34·33°/₀₀	34·3°/₀₀	34·4‰
Months	July 1948	January 1949	May 1949	June 1949	July 1949	August 1949

The results of experiments on the effect of different grades of salinity on the setting of spat under laboratory conditions are given in Table III.

TABLE III

Showing Relative Effects of Salinity Grades of Sea-water and Estuarine Water on Setting

Salinity grades with sea-water	*34·33‰ excellent	30·01°/₀₀ excellent	27·88°/₀₀ good	25·74°/₀₀ fair	21·45°/₀₀ poor	17·23°/₀₀ nil	
Salinity grades with estuarine water	..	..	*27·02°/₀₀ very poor	23·69°/₀₀ nil	20·32°/₀₀ nil	..	13·56°/₀₀ nil

* Used without dilution.

It may be concluded from the field and laboratory observations that (i) the salinity of the waters of the sea are always favourable for setting, the intensity of setting being dependent on the intensity of occurrence of larvæ of setting size, and (ii) conditions in the backwaters are favourable for setting only when the bar is open and the sea-water mixes with the back-water, so as to raise the salinity of the latter to about 28–30·0°/₀₀. It may be seen from Table III that there is a marked difference in setting in samples of water of approximately the same grades of salinity (i) when prepared with sea-water and (ii) with estuarine water when the bar is closed, preference being shown by the larvæ to settle in grades prepared with the former. Both in the backwaters and the coastal waters of Madras the pH varied only very slightly between 8·3 and 8·6 and it cannot therefore be regarded as the deciding factor in the success or failure in the setting of

TABLE IV

Larvæ and Spatfalls with Correlated Oservations in Adyar Backwaters and Madras Harbour

Dates	Adyar River Mouth and Backwaters					Madras Harbour and the adjoining sea			
	Salinity °/₀₀	Average Max. temp.°C.	Spawning	Freq. of larvæ	Setting of spats	Salinity °/₀₀	Average Max. temp.°C.	Freq. of Larvæ	Setting of spats
March 1948									
1– 7 ..	..	..	..	..	..	34·56	..	..	..
8–14 ..	20·32	29·0	f. & m.	..	N.S.	34·42	27·8	..	..
15–21 ..	..	29·8	f. & m.	0·1*	N.S.	36·97	28·0	1, 1	N.S.
22–31 ..	..	32·0	f. & m.	0·1*	N.S.	34·36	28·0	1	N.S.
April 1948									
1– 7 ..	..	..	f. & m.	0·1*	N.S.	34·58	28·2	3	N.S.
8–14 ..	..	32·8	f. & m.	0·1*	N.S.	34·74	29·0	3	N.S.
15–21 ..	..	..	..	..	..	37·25	29·0	2	N.S.
22–30 ..	28·59	31·7	nil	0·1*	N.S.	35·61	28·0	3	N.S.
May 1948									
1– 7 ..	..	..	nil	0·1*	N.S.	35·06	29·6	3	..
8–14 ..	30·23	31·4	nil	..	..	35·06	29·0	2	..
15–21 ..	..	..	nil	0	N.S.	35·33	28·8	..	..
22–31 ..	29·67	31·6	nil	..	N.S.	..	29·1	..	F.S.
June 1948									
1– 7 ..	..	..	nil	0	N.S.	34·60	29·1	3, 4	F.S.
8–14 ..	31·15	30·0	nil	0	N.S.	34·88	29·6	5, 3	..
15–21 ..	..	..	nil	0	N.S.	35·71	29·4	3, 3	M.S
22–30 ..	35·14	30·2	nil	0	N.S.	34·92	29·0	4	M.S.
July 1948									
1– 7 ..	38·47	..	nil	0	N.S.	34·93	28·6	4 5	M.S.
8–14 ..	..	28·2	nil	..	N.S.	34·79	27·8	3	..
15–21 ..	33·01	..	nil	0	N.S.	34·89	27·3	5, 5	G.S.
22–31 ..	..	27·7	nil	0	N.S.	34·60	26·7	4	G.S.
August 1948									..
1– 7 ..	34·07	28·0	nil	0	N.S.	34·74	28·0	..	N.F.S.
8–14 ..	34·43	..	nil	..	N.S.	34·65	28·2	1	..
15–21 ..	31·91	..	nil	0	N.S.	34·76	28·2	..	N.S.
22–31 ..	34·14	27·7	nil	0	N.S.	34·86	27·4	1	
September 1948									
1– 7 ..	..	26·8	nil	0	N.S.	34·76	26·3	..	..
8–14 ..	31·74	..	nil	0	N.S.	34·43	26·9	1	N.S.
15–21 ..	..	..	..	..	N.S.	34·72	26·7	0	..
22- 30 ..	21·69 23·95	26·7	..	..	..	34·73	26·8	0, 0	N.S.
October 1948									
1– 7 ..	..	26·6	..	..	..	34·21	26·4	0, 0	..
8–14 ..	26·80 23·71	..	..	0	N.S.	29·26	26·6	0	..
15–21 ..	..	26·4	nil	0	N.S.	28·71	26·4	..	..
22–31 ..	24·56	..	nil	0	N.S.	27·64	26·9	2, 0, 3	N.S.
November 1948									
1– 7 ..	..	25·8	nil	0	N.S.	28·27	26·2	3	N.S.
8–14 ..	25·08	..	nil	0	N.S.	26·01	26·0	3	N.S.
15–21 ..	..	..	nil	0	N.S.	24·79	26·8	.	N.S.
22–30 ..	23·84	26·4	f. & m.	0·1*	N.S.	24·60	26·6	3, 5, 0, 3	N.S.

TABLE IV—(*Contd.*)

Dates	Adyar River Mouth and Backwaters				Madras Harbour and the adjoining sea				
	Salinity °/₀₀	Average Max. temp.°C.	Spawning	Freq. of larvæ	Setting of spats	Salinity °/₀₀	Average Max. temp.°C.	Freq. of Larvæ	Setting of spats
December 1948									
1— 7 ..	22·39	25·0	f. & m.	0·1*	N·S.	24·49	25·1	3	N.S.
8—14 ..	..	do	f. & m.	0·1*	N.S.	26·79	25·0	..	F.S.
15—21 ..	22·52	24·0	f. & m.	·1*	N.S.	..	24·4	0, 1*	F.S.
22—31 ..	24·89	do	f. & m.	2·1*	F.S.	25·98	24·0	4	F.S.
January 1949									
1— 7 ..	..	24·2	f. & m.	4·1*	M.S.	28·39	24·5	4, 5, 3	G.S.
8—14 ..	..	do	nil	4·1*	M.S.	28·77	24·4	5	G.S.
15—21 ..	29·01	25·2	nil	4·	G.S.	29·07	25·1	4, 5	G.S.
22—31 ..	27·32	do	nil	..	G.S.	32·09	25·1	1, 1, 1	N.F.S.
February 1949									
1— 7 ..	28·87	25·0	nil	2	G.S.	32·80	25·6	0, 0	..
8—14 ..	..	do	nil	0	N.F.S.	32·82	26·6	1, 3	N.F.S.
15—21 ..	30·01	26·8	nil	..	N.F.S.	33·15	26·6	0	N.F.S.
22—28 ..	..	do	nil	0	N.F.S.	33·47	27·0	4, 1	..
March 1949									
1— 7 ..	33·9	27·0	nil	0	N.F.S.	33·91	27·2	1, 1*	N.S.
8—14 ..	..	do	nil	0	do	33·91	27·6	1, 1	..
15—21 ..	..	29·2	nil	0	do	34·01	27·9	0	..
22—31 ..	32·45	29·2	m	0	do	34·31	28·1	1	..
April 1949									
1— 7 ..	33·53	30·5	m.	0	N.S.	34·87	28·2	1, 1	N.S.
8—14 ..	..	do	nil	0	do	36·65	28·2	..	..
15—21 ..	..	31·8	do	0	do	34·60	28·3	..	N.S.
22—30 ..	35·55	do	do	0	N.S.	35·01	28·7	2	do
May 1949									
1— 7 ..	..	30·6	m.	..	N.S.	35·27	28·4	3, 3	..
8—14 ..	37·94	do	nil	0	N.S.	34·24	28·8	3	..
15—21 ..	..	29·9	m.	0	do	33·21	29·1	3, 1	G.S.
22—31	33·71	do	m.	0	N.S.	33·39	29·4	4, 5	G.S.
June 1949									
1— 7 ..	..	30·0	..	0	N.S.	31·16	28·9	1, 3	..
8—14 ..	..	do	nil	..	do	34·33	29·0	..	G.S.
15—21 ..	..	28·0	nil	..	do	34·31	29·1	2, 2	..
22—30 ..	25·3 26·01	do	nil	..	do	34·26	29·5	1, 3, 2	F.S.
July 1949									
1— 7 ..	..	28·6	..	..	N.S.	34·46	28·9	..	M.S.
8—14 ..	27·02	..	nil	0	do	34·36	28·7	2, 1, 4	G.S.
15—21 ..	..	29·4	..	..	do	34·25	30·1	4	G.S.
22—31 ..	..	do	nil	0	N.S.	34·3	30·0	4, 4	G.S.
August 1949									
1— 7 ..	28·6	29·4	nil	0	N.S.	34·40	29·5	3	G.S.
8—14 ..	..	do	nil	0	do	..	..	4	G.S.
15—21 ..	..	..	..	..	..	..	..	..	..
22—31 ..	..	..	..	..	..	..	..	..	..

Spawning.—m. male spawning; f. & m., male and female spawning.

Frequency of Larvæ.—0, absent; 1. very few; 2. few; 3. fair; 4. large numbers; 5. abundant. Nos. 0–5 indicate larvæ of setting size; 1*. larvae of straight hinge stage (small larvæ).

Spats.—*N.S.*, No spat; *F.S.*, Few spat, *N.F.S.* No fresh spat; *M.S.*, Moderate spat; *G.S.*, Good spat.

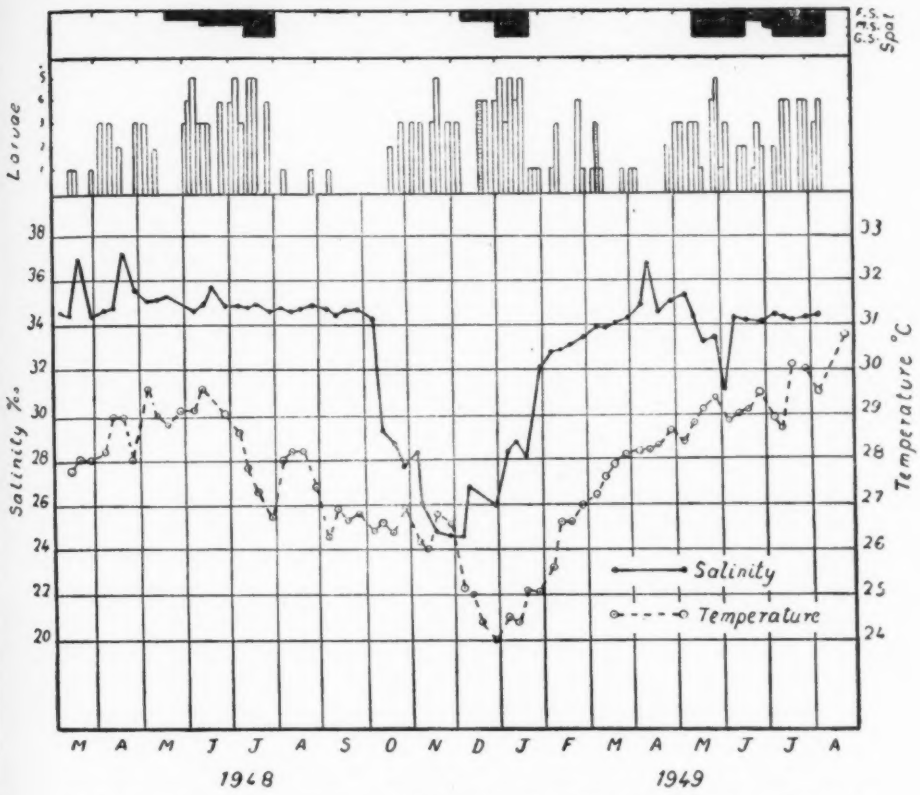

FIG. 2. Frequency of occurrence of larvæ and spatfall of *Ostrea madrasensis* in relation to variations in salinity and temperature in Madras Harbour. Data presented in Table IV. (Explaantion of lettering is as for Table IV: histograms with cross stripes indicate larvæ of young size, and those without cross stripes represent larvæ of setting stage.)

spat. Thus there still seems to be an unascertained factor in the sea-water apart from salinity which induces spatfall.

8. GENERAL CONSIDERATIONS

What seems to be of the utmost importance in the cultivation of oysters is a knowledge of the factors that induce spawning, control larval development and promote setting. It is well known that a rise in temperature of the waters in the summer months is the chief stimulating factor in inducing the European and American oysters, *O. edulis* and *O. virginica* to spawn in temperate waters. Stafford (1913), Nelson (1921, 1928 *a* and *b*), Churchill (1920), Prytherch (1929) and Galtsoff (1930, 1932) have shown that spawning

does not start in the American oyster, until the minimum or critical temperature of 20°·0 C. is reached. Even the maturing process of the gonads seems to be governed by the temperature factor: thus Nelson (1928 *b*) has shown that sperm production begins at about 10° C., deposition of yolk at 15° C. and maturation of the egg at 18° C. with wide individual variations. He (1921) has also recorded that if the temperature of the water fails to rise to the required minimum the reproductive elements are absorbed instead of being liberated. Prytherch (1929) has observed spawning at 20° C. at high tide when the pH factor is favourable, but not at low tide when, even if the temperature is high, the spawning reaction does not take place because of very low pH. Thus factors other than temperature seem to govern spawning. Galtsoff (1930, 1932, 1938 *b*) has shown that female oysters spawn not only spontaneously at 24°·5 C. but also by stimulation in the presence of sperm at a much lower temperature, *i.e.*, between 18°·6 and 20°·5 C. Hence in any locality with temperature of water at about 20° C. the females begin to spawn if the stimulus of male spawning is present. He also observed that males respond immediately to egg suspension in water as well as to sperm with a "latent period". Orton (1926 and 1937) has observed *O. edulis* breeding at 15° C. Changes in salinity have not been known to induce spawning in the American or the European oysters. Under tropical conditions of our coasts the water temperatures of the sea or the backwaters are maintained high throughout the year and do not even fall at any time below the optimum requirement of the oysters mentioned above. Hornell (1910 *a*) thought that a fall in density of the waters due to heavy rains of the north-east monsoon was the chief stimulating factor in the spawning of the Madras oyster in the east coast backwaters of South India. Experiments conducted by him in 1908 with spat collectors in Ennore during the north-east monsoon in October–December led him to the conclusion that the oysters spawned in October and November under the influence of low salinity conditions, but no records of salinity were maintained during the period of observations. He (1922) also observed that the chief spawning period was in August-September followed by supplementary spawning in March–April. My observations corroborate those of Hornell (1910, 1922) except that spawning after the north-east monsoon may extend up to about the first week of January, and that both the main and supplementary spawning periods are governed by the salinity factor in the environment. In the present investigations the salinity in the backwaters when spawning was observed, varied between 20·3 and 28·00‰, which is almost the same as the optimum requirement (22–26‰) for the developing eggs under laboratory conditions. This optimum may be attained either by a rise or fall

in salinity of the backwater; if the salinity is very high due to evaporation of water when the bar is silted up, it is brought down by the influx of flood water due to rains; or if the salinity of the backwaters is considerably low for any length of time, it is raised to the optimum requirement either by evaporation or by the opening of the bar. When the optimum salinity is thus reached spawning takes place in the backwaters, provided the gonads are ripe.

Hopkins (1931) found correlation between the periods of setting and periods of high salinity in *Ostrea virginica* of Galveston Bay, Texas, and considered that the larvæ depended in some manner directly or indirectly, on a salinity of about 20·0‰ as a stimulus to develop to setting stage. Gaarder (1932, 1933) and Gaarder and Bjerkan (1934) found that a salinity of 24‰ and above essential with 30-35‰ as the optimum for the successful growth of the larvæ of *O. edulis*. The laboratory experiments and field observations in Adyar and Ennore show that not only higher salinities up to 29–30‰, but also the mixture of the sea-water with the estuarine water as a consequence of the bar remaining open, are essential for successful larval development and spatfall in the backwaters. Higgins (1938), in the Long Island Sound, U.S.A., and Korringa (1941) in Oosterschelde, Holland, found the small salinity variations of no importance in determining success or failure of spatfall. In the small estuaries and backwaters of our coasts, however, periodical inflow and outflow of tidal and flood waters respectively with the tidal amplitude as small as three feet influence the fluctuations in salinity, and consequently of the spawning and setting of the oysters. Prytherch (1934) found larvæ of *O. virginica* undergoing complete fixation in 12 to 19 minutes in salinities between 16 and 18·5‰, but in salinities above or below this range, the duration of fixation was prolonged. He held that the alteration in the physical properties of the byssal fluid due to the changes in salinities of higher and lower grades as the cause of the delayed setting. The same author (1931 and 1934) observed in Milford Harbour abundant setting of the same larvæ in low water, which he attributed to slight variations in the copper content of the waters in the course of the tidal cycle, a view which received little support from Nelson (1931), Gaarder (1932, 1933) and Korringa (1941). All that can be said at present is that some unknown factor present in the sea-water has something to do with larval development and setting, but absent in the estuarine water which remains unconnected with the sea for a fairly long period when the bar is closed.

Butler (1949) observed inhibition of gametogenesis in 90% of *O. virginica* population in a low salinity area until the salinity rose to 6‰. This

suppression of the gonadic activity he attributed to the inability of oysters to feed under low salinity conditions. During the period of the present observations no fall in salinity in the backwaters was observed to such an extent as to cause any deleterious effect on the gonadic development.

The subject of periodicity in the breeding of marine animals in the tropics has attracted the attention of various workers, giving rise to the expression of a great variety of views. Semper (1881) and Orton (1920) held that the breeding behaviour of marine invertebrate animals is different under the tropical and temperate regions, with a tendency to be continuous in one and periodic in the other, respectively. Galtsoff (1933) thought that definite periodicity could be noticed in the breeding of animals both under tropical and temperate conditions. Anne Stephenson (1934) found in the animals of the Low Isles of the Australian Great Barrier Reef, continuous and discontinuous breeding, the latter sometimes favoured by lunar phases. Her observations find support in those of Paul (1942) on the breeding of the Madras Harbour animals. Panikkar and Aiyar (1939) in the brackish-water animals of Madras also observed different types of both continuous and discontinuous breeding, the latter in some cases determined by the rains. The present observations on the Madras Harbour oyster show that it breeds throughout the year, with two peak periods of maximum sexual activity in November–December and May–August as against the single peak in April-October observed by Paul (1942). The two peak periods seem to correspond to those of low salinity and high temperature of the coastal waters. These peaks of breeding activity under marine conditions seem to correspond with the restricted breeding periods in November-December and March–April in the backwaters.

9. Summary

1. A record of salinity of the waters of the Adyar estuary and back-water and of the Madras Harbour was maintained from March 1948 to August 1949 to study the effect of varying salinities on the breeding of the oyster, *Ostrea madrasensis*.

2. Regular examination of the gonads and observation of the act of spawning in the oyster show that under backwater conditions sexual activity is restricted to the November–December period usually followed by a supplementary spawning in March–April.

3. The occurrence of oyster larvæ of *Ostrea madrasensis* in the coastal waters of Madras near the Harbour throughout the year indicates that breeding is continuous.

4. Experiments with fertilised oyster eggs placed in varying prepared grades of salinity in the laboratory show that the optimum range of salinity necessary for early development up to the shelled veliger is from 22 to 26°/$_{oo}$ at a temperature of 26°·0–28°·5 C. The maximum and minimum salinities required for the formation of the early veligers are 36°/$_{oo}$ and 16°/$_{oo}$ respectively. The ranges of salinity from 28–36°/$_{oo}$ and 20–16°/$_{oo}$ are described as the high and low salinity ranges respectively for the formation of the early veligers.

5. Successful spawning of both sexes seems to take place in the backwaters in almost the same range as the optimum requirement for the developing eggs.

6. In the Madras Harbour the oysters breed for most part of the year in the high salinity range for the formation of early veligers. Fall in salinity in October to about the optimum range is followed about a month later by increased number of larvæ in the plankton of the waters.

7. The difference in the breeding behaviour of the oysters in the backwaters in the two consecutive March-April seasons of 1948 and 1949 is attributed to the differences in salinity, only stray cases of male spawning having been observed under high temperatures and salinities in March to May 1949.

8. The incidence of oyster larvæ in coastal waters seems to depend on two different factors, *e.g.*, (i) high temperatures in summer months and (ii) fall in salinity after the rains of north-east monsoon.

9. The peaks in the occurrence of the larvæ in the sea seem to coincide with the restricted breeding periods in the backwaters.

10. After the early development of the eggs, a rise in salinity in the backwaters to 28°/$_{oo}$ and above seems to be congenial for larval growth and spatfall.

11. The salinities which prevail in the sea seem to be always favourable for setting of spat judging from the fact that larvæ obtained from the plankton readily set in pure sea-water.

12. In the Adyar backwaters spatfall occurs only when the bar is open admitting sea-water into the estuary. There is marked difference in the intensity of setting of planktonic larvæ in two different samples of water of the same grade of salinity prepared (i) out of the sea-water from the open sea and (ii) out of the estuarine water when the bar is closed. This postulates presence of some suitable chemical or other factors in fresh sea water which promotes spatfall.

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11. References

Amemiya, I. .. .. "Observations on the early stages of development of the oyster and the effect of salinity upon it," *Suisan Gakkwai Hô.*, 1921. III, pp. 150–81. English abstract in *Jap. Jour. Zool.*, 1922, **1**, Abstract No. 121, p. 34.

—— .. .. .. "Notes on experiments on the early developmental stages of the Portuguese, American and English native oysters with special reference to the effect of varying salinity," *Jour. Mar. Biol. Assn.*, N.S., 1926, **14**, 161–75.

—— .. .. .. "Ecological studies of Japanese oysters with special reference to the salinity of their habitats," *Jour. Coll. Agric. Imp. Univ., Tokyo*, 1928, **9**, 334–82. Review by Sherwood, H. P., *Jour. Du Conseil.*, 1928, **3**, 407–09.

Annandale, N. and Kemp, S. .. "'Family Ostreidae' from Fauna of Chilka Lake," *Mem. Ind. Mus.*, 1916, **5**, 348–49.

Awati, P. R. and Rai, H. S. .. "*Ostrea cucculata* (The Bombay Oyster)," *Indian Zool. Memoirs*, 1931, **3**, 107.

Butler, P. A. .. .. "Gametogenesis in the oyster under conditions of depressed salinity," *Biol. Bull.*, 1949, **96**, 263–69.

Churchill, E. P. .. .. "The oyster and oyster industry of the Atlantic and Gulf Coasts". Appendix viii, *Rept. U.S. Comm. Fish, U.S. Bur. Fish.*, 1919 (1920), Document No. 890, 51.

*Clark, A. E. .. .. "Effects of temperature and salinity on the early development of the oyster," *Progr. Rep. Atl. Biol. Stat.*, St. Andrews, 1935, N.B. No. 16, 10.

Cole, H. A... .. .. "A system of oyster culture," *Jour. Du Conseil*, 1938, **13**, 221–35.

—— .. .. .. "Further experiments in the breeding of oysters in tanks," *Fish. Invest. Ser.* 2, 1939, **16**, No. 4, London.

*Gaarder, T. .. .. "Untersuchungen uber Produktions und Lebensbedingungen in Norwegischen Austernpollen," *Bergens Museums Arbok*, 1932, *Naturv.*, Rekke. No. 3.

—— .. .. .. "Austernzucht in Norwegen, Chemisch Biologische untersuchungen in Norwegischen Austernpollen," *Intern. Revue d. gesammt. Hydrobiol. U. Hydrogr.*, 1933, **28**, Heft 3-4, 250–61.

*Gaarder, T. and Bjerkan, P. .. *østers og østerkultur i Norge*. *Bergen*, 1934. A. S. John Greig.

Galtsoff, P. S. .. .. "The role of chemical stimulation in the spawning reaction of *Ostrea virginica* and *Ostrea gigas*," *Proc. Nat. Acad. Sci., Washington*, 1930, **16**, 555–59.

—— .. .. .. "Spawning reactions of three species of oysters," *Jour. Wash. Acad. Sci.*, 1932, **22**, 65–69.

—— .. .. .. "Factors governing the propagation of oysters and other marine invertebrates," *Proc. Fifth Pacific Sci. Cong.*, Canada, 1933, **5**.

—— .. .. .. "Physiology of reproduction of *Ostrea virginica*. I. Spawning reactions of the female and male," *Biol. Bull.*, 1938, **74**, 461–486.

—— .. .. .. "Physiology of reproduction of *Ostrea virginica*. II. Stimulation of spawning in the female oyster," *Ibid.*, 1938 *b*, **75**, 286–307.

*Higgins, E. .. .. Progress in Biological Inquiries, Append. to the *Rep. Comm. of Fish., Washington*, 1933.

Hopkins, A. E. .. .. "Factors influencing the spawning and setting of oysters in Galveston Bay, Tex.," *U.S. Bull. Bur. Fish.*, 1931, **47**, 57–83.

Hornell, J. .. .. "Note on an attempt to ascertain the principal determining factor in oyster spawning in Madras backwaters (Madras Fish. Investigations, 1908)," *Madras Fisheries Bur. Bull.*, 1910 *a*, **4**, 25–31.

—— .. .. .. "The practice of oyster culture at Arcachon (France) and its lessons for India," *Ibid.*, 1910 *b*, **5**.

—— .. .. .. "The common molluscs of South India," Report iv of 1921 (Madras Fish. Inves., 1921), *Madras Fish. Bur., Bull.*, 1922, **14**, 97–215.

Korringa, P. .. .. "Experiments and observations on the swarming, pelagic life and setting of the European flat oyster, *Ostrea edulis* L." *Arch. Neerland. De Zool.*, 1941, Tome **5**, 1–249.

Loosanoff, V. L. .. .. "Seasonal gonadal changes in the adult oysters, *Ostrea virginica* of Long Island Sound," *Biol. Bull.*, 1942, **82**, 195–206.

—— and Engle, J. B. .. "Spawning and setting of oysters in Long Island Sound in 1937, and discussion of the method for predicting the intensity and time of oyster setting," *Bull. U.S. Bur. Fish., Washington*, 1940, **49**, 217–55.

Moses, S. T. .. .. "A preliminary report on the anatomy and the life-history of the common edible backwater oyster, *Ostrea madrasensis*," *Jour. Bomb. Nat. Hist. Soc.*, 1928, **32**, 548–52.

*Nelson, T. C. .. .. "Aids to successful oyster culture. I. Procuring the seed," *New Jersey Agric. Exp. Stat. Bull.*, 1921, No. 351.

—— .. .. .. "On the distribution of critical temperatures for spawning and for ciliary activity in bivalve molluscs," *Science*, 1928 *a*, **67**, 220–21.

—— .. .. .. "Relation of spawning of the oyster to temperature," *Ecology*, 1928 *b*, **9**, 145–54.

Orton, J. H. .. .. "Sea-temperature, breeding and distribution in marine animals," *Jour. Mar. Biol. Assn., U.K.*, 1920, **12**, 339–66.

—— .. .. .. "On lunar periodicity in spawning of normally grown Falmouth oysters in 1925, with comparison of spawning capacity of normally grown and dumpy oysters," *Ibid.*, 1926, **14**, 119–225.

—— .. .. .. *Oyster Biology and Oyster Culture* (The Buckland Lectures for 1935). Edward Arnold & Co., London, 1937, p. 211.

Panikkar, N. K. and Aiyar, R. G. "The brackish-water fauna of Madras," *Proc. Ind. Acad. Sci.*, 1937, **6**, Sec. B, 284–337.

—— .. .. .. "Observations on breeding in brackish-water animals of Madras," *ibid.*, 1939, **9**, Sec. B, 343–64.

Paul, M. D. .. .. "Studies on the growth and breeding of certain sedentary organisms in the Madras Harbour," *ibid.*, 1942, **15**, Sec. B, 1–42.

Preston, H. B. .. .. "Report on a collection of mollusca from the Cochin and Ennur backwaters," *Rec. Ind. Mus.*, 1916, **12**, 26–39.

Prytherch, H. F. .. .. "Investigations of the physical conditions controlling spawning of oysters and the occurrence, distribution and setting of the oyster larvæ in Miford Harbor, Connecticut," *Bull. U.S. Bur. Fish.*, 1929, **44**, 429–503.

—— .. .. .. "The role of copper in the setting and metamorphosis of the oyster," *Science*, 1931, **73**, 429–31.

—— .. .. .. "The role of copper in the setting metamorphosis and distribution of the American oyster," *Ostrea virginica*," *Ecological Monographs* N.C., 1934, **4**, 49–107.

Roughley, T. C. .. .. "The life-history of the Australian oyster (*Ostrea commercialis*)," *Proc. Linn. Soc., N. S. Wales*, 1933, **58**, 279–333.

Semper, K. .. .. "Animal life," *Int. Science Ser., London*, 1883.

*Seno, H., Hori, J. and Kusakabe, D. "Effects of temperature and salinity on the development of the eggs of the common Japanese oyster, *Ostrea gigas*, Thunberg," *Jour. Imp. Fish. Inst., Tokyo*, 1926, **22**, 41–47.

Stafford, J. .. .. "The larvae and spat of the Canadian oyster," *American Naturalist*, 1909, **43**, New York, 31–47.

—— .. .. .. "The Canadian oyster, its development, environment and Culture," *Comm. of Conservation*, Canada, Ottawa, 1913, 159.

Stephenson Anne .. .. "The breeding of reef animals, Pt. II. Invertebrates other than corals," *Sci. Rep. Great Barr. Reef Exped.*, 1928–29, 1934, **3**, 247–72.

Sundar Raj, B. .. .. *Administration Repts., Madras Fisheries for years* 1929–30, 1930, 26–28.

Winckworth, R. .. .. "Mollusca from the Pulicat Lake," *Proc. Malacol. Soc., London*, 1931, **19**, 188–89.

* References marked with an asterisk have not been consulted in the original.

LACTIC ACID PRODUCTION IN UNSTRIATED MUSCLE AND ITS RELATION TO TONUS AND INHIBITION

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Lactic acid production in unstriated muscle has been studied by several workers (Saiki, 1908; Lovatt Evans, 1925; Rosenthal and Lasnitzki, 1928; Haarmann, 1932; Prasad, 1935). The object of the present research has been to study the production of lactic acid during tonic contraction and inhibition of unstriated muscle, and to study the effect of hydrogen ions on the formation of lactic acid.

The oxygen consumption of unstriated muscle has been measured by Meyerhof and Lohmann (1926), Rosenthal and Lasnitzki (1928), Lovatt Evans (1926), Glaister and Kerly (1936) and Rao and Singh (1940). Lovatt Evans did not find any increase in the oxygen consumption during tonic contraction. Rao and Singh found that there are two kinds of tonic contraction; during one kind the oxygen consumption increases and during the other kind, decreases. It was also found that corresponding to the tonic contractions, there are two kinds of inhibition; during one kind the oxygen consumption increases and during the other kind, decreases.

Unstriated muscle shows two kinds of tone, one, which is susceptible to asphyxia or cyanide and the other which is resistant; tonus may actually increase on asphyxiation or treatment with cyanide. Similarly glucose may increase or decrease tone, and iodoacetic acid may have opposite actions (Singh, 1937, 1949; Singh and Singh, 1946, 1947, 1948 *a*, *b*). These two kinds of tone are also shown by the nature of relaxation, which may be active or passive (Singh and Singh, 1948 *c*, 1949 *a*, 1950 *a*). The twitch contraction and lactic tone on one hand and alactic tone on the other, are subserved by different contractile mechanisms (Singh and Singh, 1949 *b*, 1950 *b*, 1950 *e*).

Singh and Singh (1949 *c*) found that glucose relieved asphyxial arrest in acid solutions. In unstriated muscle, there are several metabolic mechanisms (Singh, 1949; Singh and Singh, 1947, 1948 *a*, *b*, 1949 *d*, *e*, *f*, 1950 *d*).

Methods

These experiments were mostly performed on frog's stomach muscle from *Rana tigrina*; a few were performed on dog's stomach muscle. Frogs were killed with a blow on the head and the dogs were chloroformed. Further treatment depended upon the kind of experiment to be performed.

For determination of lactic acid in fresh muscle and in acid solutions, the entire viscus from the frog and piece of stomach near the pyloric end from the dog were immediately placed in ice-cold saline (temperature 0–3° C.) and the muscle was separated from the mucosa under saline at this temperature. The tissue was immediately used for analysis. As the purpose of these estimates was to serve as controls for experiments made with surviving strips, it was not possible to use more drastic methods such as freezing with carbon dioxide snow to arrest lactic acid production during isolation of the muscle.

For determining the effect of acidity, the frog's stomach was split longitudinally into three pieces and similarly three pieces of dog's stomach muscle were used for each experiment. These three pieces were respectively soaked in ordinary saline, in saline containing sodium iodoacetate (1 in 5,000, acetate) and saline of the requisite acidity for half an hour, all the solutions being ice-cold. This was to suppress the formation of lactic acid while the iodoacetate and hydrogen ions were diffusing into the muscle. The muscle soaked in iodoacetate served as a control to compare the depression of lactic acid formation by the acid saline.

For determining the production of lactic acid during tonic contraction and inhibition, cooling had to be avoided as it interfered with the subsequent response. In all experiments a control muscle was used. In all cases sufficient muscle was isolated to serve for the experiment and for the control. The frog's stomach muscle was split longitudinally into two or three pieces, for if transverse pieces were taken, the pyloric and cardiac halves did not behave similarly. From the dog's stomach, both sets of muscles were obtained from adjacent regions.

The muscles were dried gently between moist filter-paper and the moist weight determined. The weight of the pieces used varied from 0·4 to 2·3 g The muscles were then soaked in various solutions for one hour, anaerobiasis being produced by bubbling hydrogen. It has been shown (Prasad, 1935) that in mammalian unstriated muscle, most of the lactic acid is formed in one hour at 37° C. Therefore the muscles were soaked at room temperature, 25–30° C.; under these conditions lactic acid production continues

for two hours, especially in frog's muscle. This was tested by four experiments, two on frog's and two on dog's muscle. Thus the lactic acid content of the first frog's muscle after 0, 1 and 2 hours soaking in saline was 2·59, 2·67 and 3·21 mg. per 100 g. of wet muscle respectively. In the second frog's muscle it was 1·59, 2·27 and 4·18 mg. respectively. In the first dog's muscle, it was 4·57, 11·46 and 12·5 mg. respectively; in the second dog's muscle, it was 5·68, 10·98 and 13·8 mg. respectively. These experiments were supplemented by those in which the muscles were soaked in solutions containing 0·2 p.c. glucose, so as to ensure continued formation of lactic acid.

The composition of the frog and mammalian saline was as described previously (Singh, 1939, 1940); the pH of the saline was 8 (borate buffer). The acid salines were made with phosphate, 15 to 30 mg. P per 100 c.c. (pH 7, 6 and 5), acetate, 0·002 *M*, (pH 4) and with hydrochloric acid (pH. 3. 2 and 1). Glucose-free solutions were used except when otherwise stated.

After soaking the muscles were ground with sand in ice-cold saline, using cold pestles and mortars. The total volume of the muscle and the saline was 8 c.c., the saline in which the muscle was soaked, being also included. After grinding, the proteins were precipitated by adding 1 c.c. of 10 p.c. sodium tungstate and 1 c.c. of $\frac{2}{3}$ *N* sulphuric acid; 2 c.c. of the protein-free filtrate was used for analysis. In case of muscles, weighing over 2 g. 10 c.c. of distilled water was added and 4 c.c. of the protein-free filtrate was used. In frog's muscle, it was found that the lactic acid content of muscles ground at room temperature and in cooled saline showed no significant difference, and so grinding in cooled saline was abandoned. Thus the lactic acid content of 5 muscles ground at room temperature was 5·28, 5·8, 2·41, 2·19 and 1·63 mg./100 g. respectively, average being 3·46 mg. The lactic acid content of control muscles, ground in cold saline was 4·32, 5·55, 2·02, 2·31 and 1·86 mg./100 g. respectively, the average being 3·21 mg. There was always a control muscle used in each experiment and only comparative values were required.

Lactic acid was determined by the method of Barker and Summerson (1941). The glucose and other interfering material of the protein-free filtrate was removed by treatment with copper sulphate and calcium hydroxide. An aliquot of the resulting solution was heated with concentrated sulphuric acid to convert lactic acid to acetaldehyde, which was then determined colorimetrically by reaction with *p*-hydroxy diphenyl in the presence of copper ions.

The above method was tested on a frog's gastrocnemius, tetanised for 5 minutes. It gave a value of 0·23 p.c. The lactic acid content of muscles in this paper are given in mg. per 100 g. of wet muscle.

Results

Lactic acid in fresh muscle.—The lactic acid content of fresh frog's muscle is low compared to that of striated muscle. Thus the lactic acid content of 6 frog's muscles was 0·33, 1·5, 1·3, 1·26, 0·36 and 0·57 mg./100 g. respectively, the average being 0·89 mg. or 0·001 p.c. The lactic acid content of 3 dog's muscles was 4·57, 5·68 and 3·24 mg., average 3·37 mg. or 0·003 p.c. This figure is lower than that found by other observers. It is probably due to the fact that their estimations were mostly on gut muscle which shows predominantly lactic tone; dog's muscle mostly shows alactic tone. The fact that the lactic acid content of striated muscle is higher than that of unstriated muscle, shows that the metabolism of tonus is less than that of relaxed state, in agreement with previous findings.

Effect of soaking.—If the muscle is soaked in saline, the lactic acid content increases, even if supplied with oxygen. Thus in the above series of experiments, in which the lactic acid content of fresh frog's muscles is given, that of 6 control muscles soaked in saline for one hour and oxygenated, was 2·9, 2·3, 2·9, 3·6, 1·06 and 1·01 mg. respectively, average 2·3 mg., an increase of 155 p.c. This increase is probably due to lactic tone of the muscle.

The formation of lactic acid, as a result of soaking in saline would deplete the energy reserves of the muscle. This might perhaps account for the diminution of excitability which occurs with soaking. This is supported by the fact that dog's muscle, in which the production of lactic acid is more rapid, shows greater loss of excitability than frog's muscle. It was noticed previously that active relaxation diminished as a result of soaking (Singh and Singh, 1949 *a*).

Effect of glucose.—Glucose, 0·1-0·2 p.c. increases lactic tone of both oxygenated and asphyxiated muscles (Singh, 1949); lactic acid production is also increased. Thus the lactic acid content of 6 asphyxiated frog's muscles soaked in saline for one hour was 5·08, 5·52, 5·28, 4·42, 5·39 and 4·94 mg. respectively, average 5 mg.; that of control muscles soaked in 0·2 p.c. glucose was 6·94, 6·79, 6·84, 12·49, 13·36 and 13·02 mg. respectively, average 10 mg., an increase of nearly 100 p.c.

Iodoacetic acid decreases lactic tone (Singh, 1949; Singh and Singh, 1949 *a*, fig. 11) and also suppresses the formation of lactic acid in alkaline

(Tables I, II, IV) and in acid solutions. Thus the lactic acid content of 3 asphyxiated frog's muscles soaked for one hour in saline of pH 5 was 7·35, 5·15 and 8·69 mg. respectively, average 7·1 mg.; that of control muscles soaked in 1 in 5,000 sodium iodoacetate was 1·75, 1·35 and 2·19 mg. respectively, average 1·8 mg.

Effect of tonic contraction

These experiments were divided into two groups. In the first group the lactic acid formation during normal tone was determined. In the second group the muscle was stimulated with potassium.

Effect of normal tone.—It has been shown that the pyloric and the cardiac halves of the frog's stomach respectively exhibit alactic and lactic tones (Singh and Singh, 1950 *b*). They also differ in their carbohydrate metabolism. Thus the lactic acid content of the pyloric halves of 9 asphyxiated frog's muscles immersed in saline for one hour, was 3·38, 3·95, 3·57, 3·07, 2·78, 2·78, 3·08, 3·88 and 7·14 mg. respectively, average, 3·73 mg.; that of the corresponding cardiac halves was 5·36, 6·34, 5·71, 4·07, 4·95, 4·29, 5·00, 7·50 and 8·33 mg. respectively, average 5·73 mg.

The lactic acid content of the pyloric half was still less than that of the cardiac half, even when the former was exerting a tension of about 50 g. Thus the lactic acid content of the pyloric halves of such 9 asphyxiated frog's muscles immersed for one hour was 4·84, 5·72, 9·37, 5·77, 9·98, 9·03, 6·09, 9·21 and 6·62 mg. respectively, average, 11·11 mg., that of the corresponding cardiac halves, exerting no tension was 7·63, 7·32, 9·26, 9·11, 9·97, 11·16, 8·93, 11·18 and 12·46 mg. respectively, average, 14·5 mg.

Effect of potassium.—Potassium produces a tonic contraction of dog's stomach muscle. The lactic acid formation slightly decreases. Thus the lactic acid content of 6 asphyxiated dog's muscles, immersed in saline for one hour was 22·17, 20·13, 19·56, 13·93, 14·29 and 14·06 mg. respectively, average 17·36 mg.; that of corresponding muscles immersed in saline, 40 p.c. sodium of which had been replaced with potassium, was 17·69, 20·78, 15·56, 11·76, 13·13 and 13·41 mg. respectively, average 15·39 mg.

In frog's muscle, potassium produces only a transient contraction owing to rapid accommodation, and hence, no significant effect on the lactic acid formation was found. Thus the lactic acid content of 8 oxygenated frog's muscles immersed in saline for one hour, was 1·70, 2·10, 1·87, 3·76, 2·97, 3·50, 1·60 and 2·97 mg. respectively, average 3·41 mg.; that of corresponding muscles in potassium was 1·30, 2·20, 1·58, 3·40, 3·75, 1·85, 2·40 and 2·20 mg. respectively, average 3·11 mg. The lactic acid content of 6

asphyxiated frog's muscles immersed in saline for one hour was 4·09, 6·43, 6·17, 5·98, 7·78 and 6·36 mg. respectively, average 6·13 mg.; that of corresponding muscles in potassium was 3·75, 7·74, 5·24, 5·60, 7·74 and 6·00 mg. respectively, average 6·01 mg.

Effect of inhibition

Inhibition in frog's muscle is produced by ammonium and adrenaline. Ammonium produces only inhibition at pH 7; at pH 8, the inhibition is preceded by a transient contraction only, so that for greater part of the time, the muscle is relaxed. Rao and Singh (1940) found that at pH 7, ammonium increases the oxygen consumption, though the muscle relaxes. Similar results were found with lactic acid formation, which is increased by ammonium. Thus in 9 asphyxiated frog's muscles immersed in saline of pH 7 for one hour, the lactic acid content was 5·00, 4·07, 5·79, 5·71, 3·95, 5·42, 6·47, 6·39 and 6·16 mg. respectively, average 5·44 mg.; that of corresponding muscles in saline, 25 p.c. of the sodium of which was replaced with ammonium, was 5·35, 6·32, 6·90, 6·05, 5·03, 6·47, 8·18, 7·89 and 7·44 mg. respectively, average 6·63 mg.

During inhibition produced by adrenaline, the oxygen consumption of frog's muscle decreases (Rao and Singh, 1940); slight decreases in the lactic acid formation was also found. Thus the lactic acid content of 6 asphyxiated frog's muscles immersed in saline of pH 7 for one hour was 4·73, 7·82, 5·06, 5·49, 7·32 and 6·61 mg. respectively, average 6·17 mg.; that of corresponding muscles in adrenaline (1 in 100,000) was 6·08, 7·83, 4·86, 5·12, 5·56 and 6·35 mg. respectively, average 5·97 mg.

Effect of hydrogen ions

In skeletal and cardiac muscle, lactic acid is suppressed in acid solutions. In unstriated muscle, Singh and Singh (1949 *c*) found that glucose relieved asphyxial arrest in acid solutions. The effect of hydrogen ions was therefore tested on lactic acid formation. Lactic acid formation continues in pH 6 and 5. It begins to be suppressed in pH 4. In hydrochloric acid, it begins to be suppressed in pH 2; this is presumably due to its difficulty in penetrating of the muscle (Tables 1, 2). Added glucose is utilised in acid solutions (Table III). If frog's muscle is oxygenated, then the lactic acid formation is suppressed in acid solutions (Table IV). This suggests either better oxidation of lactic acid in acid solutions or that the mechanism of formation of lactic acid under aerobic and anaerobic conditions is different in acid solutions. This system does not appear to be present in the dog

TABLE I. *Effect of pH on the lactic acid content of asphyxiated frog's stomach muscle immersed for one hour*

Lactic acid mg./100 g. of wet muscle. I.A.A. = sodium iodoacetate

No. of Experiment	Lactic acid in pH 8	Lactic acid in I. A. A.	Lactic acid in acid solution	pH of acid solution
1	5·48	2·95	4·15	6
2	4·47	3·23	3·64	6
3	3·23	1·20	3·39	6
4	3·79	2·34	2·52	6
5	5·28	1·49	6·14	6
6	2·57	1·74	4·45	6
7	3·47	2·36	3·92	6
8	4·81	1·87	5·06	6
9	5·29	4·78	5·01	5
10	6·07	3·15	6·13	5
11	5·18	2·01	5·18	5
12	5·8	2·16	4·77	5
13	5·6	4·41	6·13	5
14	6·69	3·08	7·33	5
15	2·33	0·81	2·34	5
16	3·09	1·14	2·67	5
17	10·08	2·58	4·26	4
18	8·34	1·73	4·52	4
19	7·17	1·55	3·05	4
20	8·40	1·82	4·61	4
21	12·14	1·91	7·12	4
22	10·15	2·81	5·16	4
23	12·78	1·86	5·58	3
24	9·71	2·94	5·67	3
25	8·72	1·76	6·25	3
26	7·83	1·49	6·82	3
27	14·33	4·38	19·02	3
28	22·22	6·41	21·42	2
29	6·58	1·23	1·35	2
30	6·06	1·67	1·63	2
31	11·03	2·83	2·78	2
32	11·56	3·26	3·28	3

TABLE II. *Effect of pH on the lactic acid content of asphyxiated dog's stomach muscle immersed for one hour*

Lactic acid, mg./100 g. of wet muscle. I.A.A. = Sodium iodoacetate

No. of experiment	Lactic acid in pH 8	Lactic acid in I.A.A.	Lactic acid in acid solution	pH of acid solution
1	13·75	9·28	16·28	6
2	17·08	7·28	13·43	6
3	14·49	7·60	14·18	5
4	12·82	8·55	19·05	5
5	9·86	2·45	9·41	5
6	8·33	2·17	8·75	5
7	13·46	8·47	9·88	4
8	13·09	11·18	11·57	4
9	15·89	7·48	14·46	3
10	15·64	13·89	16·10	3
11	15·49	5·99	7·39	2
12	12·04	6·18	8·52	2

TABLE III. *Effect of pH on the lactic acid content of asphyxiated dog's and frog's stomach muscle immersed for one hour in salines containing 0·2 p.c. glucose*

Lactic acid, mg./100 g. of wet muscle

No. of experiment	Lactic acid in pH 8	Lactic acid in acid solution	pH of acid solution	Animal
1	11·36	15·38	6	frog
2	12·41	11·77	6	do
3	8·8	11·00	6	do
4	21·92	21·15	6	dog
5	19·31	19·91	6	do
6	19·99	18·85	6	do
7	8·45	7·62	5	frog
8	5·46	4·57	5	do
9	6·21	8·15	5	do
10	5·28	7·61	5	do
11	6·51	10·91	5	do
12	9·47	9·92	5	do
13	25·09	22·76	5	dog
14	24·34	24·21	5	do
15	24·88	24·09	5	do
16	5·29	4·47	4	frog
17	4·96	4·31	4	do
18	4·23	4·78	4	do

TABLE IV. *Effect of pH on the lactic acid content of oxygenated unstriated muscle immersed for one hour*

Lactic acid mg./100 g. of wet muscle. A.A.I. = Sodium iodoacetate

No. of experiment	Lactic acid in pH 8	Lactic acid in I.A.A.	Lactic acid in acid solution	pH of acid solution	Animal
1	3·16	1·19	1·57	6	frog
2	3·21	0·97	1·46	6	do
3	2·52	1·08	1·71	6	do
4	3·21	1·11	1·62	6	do
5	2·68	2·30	2·36	6	do
6	1·98	0·96	1·36	6	do
7	2·90	0·33	0·71	5	do
8	1·89	0·31	1·01	5	do
9	2·95	1·36	1·79	5	do
10	2·40	0·96	1·20	5	do
11	2·68	0·90	1·71	5	do
12	2·66	1·11	1·79	5	do
13	2·55	1·08	1·11	4·5	do
14	2·28	1·04	1·69	4·5	do
15	2·19	1·72	1·53	4·5	do
16	2·11	0·97	0·98	4·5	do
17	2·0	0·86	1·20	4·5	do
18	1·37	0·82	1·21	4·5	do
19	4·88	3·00	1·21	4	do
20	1·81	1·00	1·63	4	do
21	3·28	1·74	1·78	4	do
22	3·69	2·64	1·86	4	do
23	3·43	1·75	1·61	4	do
24	2·38	2·08	2·12	4	do
25	12·30	8·25	12·61	6	dog
26	15·85	12·05	12·50	6	do
27	16·05	8·80	15·24	5	do
28	17·96	8·63	16·01	5	do

muscle; this difference, however, may be due to greater thickness of dog's tissue so that oxygen does not diffuse in rapidly enough.

Effect of cyanide.—Cyanide does not completely abolish the activity of unstriated muscle, just as asphyxia does (Singh, 1949). Correspondingly, it was found that the lactic acid content of cyanide poisoned muscles oxygenated for one hour was less than that of corresponding asphyxiated muscles. Thus the lactic acid content of 6 asphyxiated muscles was 6·76, 7·32, 5·28, 7·39, 7·19 and 5·43 mg. respectively, average 6·56 mg.; that of the corresponding 6 muscles poisoned with sodium cyanide (1 in 5,000) was 5·36, 6·01, 5·17, 6·25, 6·36 and 5·52 mg. respectively, average 5·78 mg.

Effect of stretching.—It was found that stretching diminishes the oxygen consumption of unstriated muscle (Rao and Singh, 1940). In 3 out of 6 experiments stretching diminished the lactic acid formation of dog's muscle, asphyxiated for one hour. In one experiment it produced no significant difference; in two experiments it increased the lactic acid formation. Thus the lactic acid content of 6 stretched dog's muscles was 10·08, 10·27, 13·36, 8·33 and 7·84 mg. respectively; that of the corresponding unstretched muscles was 10·56, 8·98, 7·34, 10·18, 10·02 and 10·45 mg. respectively. Thus stretching stimulates both the lactic as well as alactic mechanisms (Singh and Singh, 1950 *b*).

Effect of electric current.—Stimulation of unstriated muscle with alternating current increases the oxygen consumption (Rao and Singh, 1940) and lactic acid production (Prasad, 1935). Prasad found that in cat's colon, the lactic acid formation increased by 12 p.c. In three experiments, it was found that the lactic acid formation of dog's muscle increased by 11 p.c. as result of stimulating with alternating current (10 volts for 10 seconds every minute) for 15 minutes.

Discussion

These experiments fully confirm the previous findings about the existence of two kinds of contraction in unstriated muscle. During one the lactic acid formation diminishes—alactic tone, and during the other it increases—lactic tone and twitch contraction. This can also be seen by the action of iodoacetic acid which diminishes or increases tone. The diminution in metabolism during one kind of tonic contraction can be predicted by the application of a principle akin to the theorem of Le Chetalier in thermodynamics. For example, a finding which can be easily demonstrated is the contraction of unstriated muscle on diminution of energy reserves, that is,

as a result of asphyxiation or fatigue; it follows, therefore, that a certain kind of contraction would lead to diminished expenditure of energy.

In some of the previous papers (Singh and Singh, 1948 *a*, 1949 *d*, e), the view of acid solutions being nonglycolytic will have to be abandoned. The metabolic mechanisms in acid and alkaline solutions, however, are different (Singh and Singh, 1949 *c*). This is also shown by the differential effect of acidity on the lactic acid formation in frog's unstriated muscle, this being suppressed under aerobic, but not under anaerobic conditions.

Summary

1. Lactic acid content of fresh frog's and dog's unstriated muscle is less than that of striated muscle; this shows that during one of the tonic contractions of unstriated muscle the metabolism is less than in the relaxed state.

2. In unstriated muscle, lactic acid is formed as a result of soaking; this is presumably due to lactic tone.

3. Glucose increases one kind of tone as well as lactic acid formation; iodoacetic acid has the opposite action.

4. The pyloric half of frog's stomach which shows alactic tone forms less lactic acid than the cardiac half which shows lactic tone.

5. The pyloric half of frog's stomach when exerting a tension of about 50 g. produces less lactic acid than relaxed cardiac half.

6. Potassium produces a tonic contraction of dog's stomach muscle, during which lactic acid formation diminishes.

7. Ammonium produces inhibition of frog's stomach muscle during which the lactic acid formation increases. Adrenaline produces slight diminution.

8. Lactic acid is formed in asphyxiated unstriated muscle in acid solutions; added glucose is also utilised.

9. In frog's muscle, lactic acid formation is suppressed in acid solutions under aerobic but not anaerobic conditions.

10. If the muscle is poisoned with cyanide, the lactic acid formation is less than when it is asphyxiated.

11. Stretching increases as well as decreases the formation of lactic acid.

12. Stimulation with alternating current for 15 minutes, increases the lactic acid formation by 11 p.c.

References

Barker and Summerson, W. H. — *J. Biol. Chem.*, 1941, **138,** 535. Quoted from Hawk, P. B., Oser, B. L. and Summerson, W. H., 1947, *Practical Physiological Chemistry*, Churchill, London.

Evans, C. L. .. .. — *Biochem. J.*, 1925, **19,** 1115.

—— .. .. .. — *Physiol. Rev.*, 1926, **6,** 358.

Glaister, D. and Kerly, M. .. — *J. Physiol.*, 1936, **87,** 56.

Haarmann, W. .. .. — *Biochem. Z.* 1932, **255,** 103. Quoted from Prasad, B. N., *J. Physiol.*, 1935, **85,** 239.

Meyerhof, O. and Lohmann, K. — *Ibid.*, 1926, **171,** 381.

Prasad, B. N. .. .. — *J. Physiol.*, 1935, **85,** 239.

Rao, M. S. and Singh, I. .. — *Ibid.*, 1940, **98,** 12.

Rosenthal, O. and Lasnitzki, A. — *Bioch. Z.*, 1928, **196,** 340.

Saiki, T. .. .. .. — *J. Biol. Chem.*, 1908, **4,** 483.

Singh, I. .. .. .. — *J. Physiol.*, 1937, **89,** 8P.

—— .. .. .. — *Ibid.*, 1939, **96,** 367.

—— .. .. .. — *Ibid.*, 1940, **98,** 155.

—— .. .. .. — *Proc. Ind. Acad. Sci.*, 1949, **29,** 190

Singh, S. I. and Singh, I. .. — *Ibid.*, 1946, **23,** 312.

———— .. .. — *Curr. Sci.*, 1947, **16,** 259.

———— .. .. — *Proc. Ind. Acad. Sci.*, 1948 *a*, **27,** 127.

———— .. .. — *Curr. Sci.*, 1948 *b*, **17,** 321.

———— .. .. — *Ibid.*, 1948 *c*, **17,** 306.

———— .. .. — *Proc. Ind. Acad. Sci.*, 1949 *a*, **30,** 343.

———— .. .. — *Ibid.*, 1949 *b*, **30,** 263.

———— .. .. — *Ibid.*, 1949 *c*, **30,** 47.

———— .. .. — *Ibid.*, 1949 *d*, **30,** 168.

———— .. .. — *Ibid.*, 1949 *e*, **30,** 215.

———— .. .. — *Curr. Sci.*, 1949 *f*, **18,** 155.

———— .. .. — *Ibid.*, 1950 *a*, **19,** 60.

———— .. .. — *Proc. Ind. Acad. Sci.*, 1950 *b*, **31,** 351.

———— .. .. — *Ibid.*, 1950 *c*, **32,** 12.

———— .. .. — *Curr. Sci.*, 1950 *d*, **19,** 219.

———— .. .. — *Proc. Ind. Acad. Sci.*, 1950 *e* (In the Press).

STUDIES ON ENZYME 'LIPASE'

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Comparative Study of Lipases from Oilseeds

Ramakrishnan ang Nevgi[1] studied the lipase extracted from castor seed. An attempt is made to analyse the different oilseeds available in India for their lipase content with a view to get cheap and active lipase on a large scale.

The lipases are prepared from the following oilseeds according to Longnecker and Haly's method[2] and the different factors which control the activity of these lipases are studied—(1) Castor (*Ricinus communis*); (2) Groundnut (*Arachis hypogea*); (3) Sesamum (*Sesamum Indiacum*); (4) Mysore seed (*Guizatia abyssinica*); (5) Safflower (*Carthamus*); (6) Mustard (*Brassica nigra*); (7) Cotton seed (*Gossypium herbaceum*).

Effect of the Nature of Buffer, Substrate and Lipase on the Activity of the Lipase

Three sets of different buffer mixtures have been prepared and the hydrolysis of freshly prepared oils by the lipases have been studied using the buffer mixtures.

Disodium phosphate-citric acid buffer mixtures of different pH are prepared according to McIlvaine's method,[3] sodium acetate-HCl and sodium acetate-acetic acid buffer mixtures of different pH according to Walpole's method.[4] The buffer mixtures are tested for their respective pH and if necessary adjusted by addition of any of the constituents of the buffer mixtures. The above prepared buffer mixtures are used for the experiments.

The oils used for the experiment are freshly prepared having the following physical constants:—

	Sap. value	Iodine value	f.f.a. for 1 c.c. of oil in terms of N/10 NaOH
Groundnut oil ..	188·6	100·1	0·05 c.c.
Sesamum oil ..	189·3	114·2	0·05 ,,
Safflower oil ..	187·2	113·4	0·03 ,,
Cotton seed oi. ..	194·5	113·2	0·10 ,,
Castor oil ..	176·7	83·7	0·30 ,,
Mustard oil ..	174·7	106·8	0·10 ,,
Mysore seed oil ..	185·6	118·6	0·05 ,,

First of all the hydrolysis of fresh groundnut oil by different lipases using disodium phosphate-citric acid buffer is carried out.

Each set of the experiments consists of 1 c.c. of the oil, 5 c.c. of water, 0·1 gm. of lipase, 2 c.c. of buffer mixture of varying pH value and a few drops of toluene in a test-tube. It is corked well, shaken thoroughly for a constant time to make its contents homogeneous and incubated for 24 hours at 37° C. Always a blank accompanied each sample. After the period of incubation each test-tube is taken out and the contents titrated against N/10 sodium hydroxide after adding 25 c.c. of neutral alcohol and warming for some time. The precautions are taken to take readings under sterile conditions. The difference between the blank and the sample in terms of c.c. of N/10 sodium hydroxide will give the activity of the lipase. The results are tabulated below.

It is observed that the castor and groundnut seed lipases are the best active lipases and the activity of the lipase increases in the order disodium phosphate-citric acid, sodium acetate-HCl and sodium acetate-acetic acid buffer.

It is found that the optimum pH changes with the nature of the lipase and substrate whereas it is almost constant with different buffer mixtures. The castor and groundnut lipases are the most active, the castor and the groundnut oil are the best substrates and sodium acetate-acetic acid buffer is the best buffer.

Effect of Buffer Concentration on the Hydrolysis of Groundnut Oil by Lipase

The hydrolysis of groundnut oil is carried out by changing the concentration of the buffer solution and keeping the other factors constant,

TABLE I

BUFFER : Disodium phosphate—Citric acid

Set No.	Substrate used	Oil in c.c.	pH value	Difference in c.c. of N/10 NaOH between the sample and the blank in case of						
				Castor	Ground-nut	Seasame	Mysore	Safflower	Mustard	Cotton seed lipase
1	Groundnut oil	1·0	3·6	6·3	5·3	2·3	4·7	2·7	5·2	1·7
2		1·0	4·8	12·2	7·2	3·1	5·2	2·9	7·0	2·8
3		1·0	5·2	7·8	11·8	4·2	7·4	3·2	5·8	4·2
4		1·0	5·5	6·4	6·8	5·3	5·8	4·8	4·9	3·1
5		1·0	5·7	5·3	5·2	4·8	4·9	6·6	4·2	2·8
6		1·0	6·2	4·2	3·9	3·2	4·2	5·2	3·8	2·6
7		1·0	7·2	3·3	2·1	2·9	3·7	4·6	3·5	1·9
8		1·0	7·6	2·7	1·7	1·8	2·3	3·8	2·9	1·2
				BUFFER : Sodium acetate—HCl						
1	Groundnut oil	1·0	3·8	9·9	6·4	3·2	4·2	4·3	6·1	4·4
2		1·0	3·9	10·8	6·9	3·7	4·9	5·0	6·5	4·9
3		1·0	4·4	13·4	7·3	4·4	5·8	5·5	6·9	5·3
4		1·0	4·8	11·2	8·5	4·7	6·4	5·9	7·2	6·2
5		1·0	5·2	10·9	12·1	5·0	7·9	6·3	8·4	5·8
6		1·0	5·6	10·9	9·1	6·4	6·2	7·2	7·0	3·4
7		1·0	5·7	9·8	8·4	6·0	5·7	6·1	6·7	3·0
8		1·0	6·0	9·1	7·2	5·1	5·1	5·8	5·8	4·3
				BUFFER : Sodium acetate—Acetic acid						
1	Groundnut oil	1·0	3·8	11·2	10·1	5·2	7·6	7·2	7·3	5·4
2		1·0	4·6	15·4	10·7	5·7	8·0	7·8	9·8	7·2
3		1·0	4·8	13·8	11·8	6·0	8·5	8·1	8·1	6·0
4		1·0	5·3	13·1	14·5	6·4	9·1	8·9	7·8	5·8
5		1·0	5·5	12·6	11·7	7·2	8·9	6·5	7·3	5·2
6		1·0	5·7	12·2	12·0	6·2	8·3	6·1	6·7	4·9
7		1·0	5·9	11·4	10·6	5·8	7·9	5·9	6·4	4·3
8		1·0	6·0	10·5	10·2	4·9	7·5	5·5	6·0	3·7

TABLE II

BUFFER : Disodium phosphate—Citric acid. Lipase : Castor seed lipase

			Castor oil	Ground-nut oil	Sesamum oil	Mysore oil	Safflower oil	Mustard oil	Cotton seed oil
1	1·0	3·6	7·8	6·3	6·1	6·5	6·3	4·8	4·2
2	1·0	4·8	13·0	12·2	8·8	7·3	7·5	5·9	5·9
3	1·0	5·2	8·9	7·8	6·5	8·1	6·9	6·8	5·0
4	1·0	5·5	7·5	6·4	5·8	7·6	6·2	5·4	4·7
5	1·0	5·7	6·9	5·3	4·7	6·5	5·8	5·0	4·2
6	1·0	6·2	5·8	4·2	3·9	5·4	5·4	4·7	3·8
7	1·0	7·2	4·7	3·3	2·8	4·9	4·9	4·3	3·2
8	1·0	7·6	3·9	2·7	1·9	3·9	4·3	3·8	2·9

Disodium phosphate-citric acid buffer of 5·2 pH is used. The amount of buffer added varied from 1 to 8 c.c.

Experiments are carried out using varying quantities of phosphate buffers. The results are given in Table III.

TABLE III

Set No.	Oil in c.c.	Buffer used in c.c.	Difference between the sample and the blank in terms of c.c. of N/10 NaOH for	
			Castor	Groundnut lipase
1	1·0	1·0	5·3	12·5
2	1·0	2·0	7·8	11·8
3	1·0	3·0	6·3	10·1
4	1·0	4·0	5·2	9·5
5	1·0	5·0	4·8	8·3
6	1·0	6·0	4·3	7·6
7	1·0	7·0	4·0	7·3
8	1·0	8·0	3·5	6·5

From the above table, it can be seen that buffer concentration plays a great roll in enzymic hydrolysis. The optimum buffer concentration is 1–2 c.c. depending upon the nature of the lipase.

Effect of Substrate Concentration on the Hydrolysis of Groundnut Oil by Lipase

The hydrolysis of groundnut oil is carried out using different lipases by changing the concentration of the oil and keeping the other factors constant in order to study the effect of substrate concentration on the enzymic hydrolysis of oil.

Experiments are carried out using varying quantities of the substrate. The results are given in Table IV.

From Table IV, it can be seen that the maximum hydrolysis is obtained when the concentrtaion of the substrate is 1–2 c.c. depending upon the nature of the lipase.

TABLE IV

Set No.	Oil in c.c.	Difference between the sample and the blank in terms of c.c. of N/10 NaOH in case of	
		Castor	Groundnut lipase
1	1·0	7·8	11·8
2	2·0	7·1	11·8
3	3·0	6·8	10·9
4	4·0	6·5	10·3
5	5·0	5·7	9·5
6	6·0	5·7	9·5
7	7·0	5·3	9·1
8	8·0	4·9	8·7

Effect of Enzyme Concentration on the Hydrolysis of Groundnut Oil by Different Lipases

The next factor is the effect of enzyme concentration on the hydrolysis of oil by different lipases. Experiment is carried out keeping all other factors constant except the concentration of the lipase.

Experiments are carried out using varying quantities of lipase. The results are given in Table V.

TABLE V

Set No.	Oil in c.c.	Enzyme in g.	Difference in c.c. of N/10 NaOH between the sample and the blank for	
			Castor	Groundnut lipase
1	1·0	0·1	7·8	11·8
2	1·0	0·2	8·8	12·1
3	1·0	0·3	10·2	15·2
4	1·0	0·4	13·2	17·4
5	1·0	0·5	13·6	18·8
6	1·0	0·6	15·6	20·1
7	1·0	0·7	17·8	23·4

It is observed that the percentage hydrolysis of the oil goes on increasing as the concentration of the enzyme increases. Hence greater is the concentration of the enzyme, greater is the activity.

Effect of Temperature on the Enzymic Hydrolysis of Groundnut Oil by Different Lipases

The next factor to be studied is the effect of temperature on the hydrolysis of groundnut oil by lipases.

Different sets of experiments are carried out by keeping the contents in the incubator at different temperatures for a fixed time and then titrating against N/10 sodium hydroxide. The hydrolysis of groundnut oil is carried out by keeping the contents in the incubator for two hours at temperatures 28° C.; 30° C.; 37° C ; 40° C.; 75° C. and 100° C. The results are given in Table VI.

TABLE VI

Set No.	Temperature °C.	Difference between the sample and the blank in terms of c.c. of N/10 NaOH	
		Castor	Groundnut lipase
1	28·0	3·9	8·1
2	30·0	4·7	8·9
3	35·0	5·0	9·2
4	37·0	5·2	9·2
5	40·0	4·8	8·6
6	75·0	3·0	4·1
7	100·0	0·0	0·0

From the above table, it can be seen that the optimum temperature for enzymic hydrolysis of oil is 35° C.–37° C.

Effect of Salts and other Organic Substances on the Enzymic Hydrolysis of Groundnut Oil by Lipase

The action of the following substances has been studied on the hydrolysis of groundnut oil by different lipases: Strychnine sulphate, albumin, ascorbic acid, sodium taurocholate, sodium acetate, strychnine chloride, glycine, citric acid, gum arabic, acetic acid, ammonium phosphate, sodium phosphate, potassium phosphate, HCl; $CaCl_2$; NaCl and $MnSO_4$.

Each set of the experiments consists of 1 c.c. of groundnut oil, 5 c.c. of water, 2 c.c. of phosphate buffer of pH 5·2, and 0·1 gm. of lipase and 0·1 gm. of substance (or 1 c.c. of solution in case of HCl and acetic acid) incubated at 37° C. for 24 hours. The blank accompanied the sample. After the period of incubation the content is titrated against N/10 NaOH after the addition of 25 c.c. of neutral alcohol and warming for some time. The difference between the sample and the blank gives the activity of the lipase in terms of c.c. of N/10 NaOH. The difference shows whether a particular salt is an accelerator or inhibitor depending upon whether the difference is positive (showing accelerating effect) or negative (showing retarding effect). The results are given in Table VII.

TABLE VII

Set No.	Name of the salt	Difference in c.c. of N/10 NaOH between the sample and the blank for	
		Castor	Groundnut lipase
1	Strychnine sulphate	+1·2	+0·8
2	Strychnine chloride	+3·1	+2·5
3	Albumin	−0·3	−0·5
4	Sodium taurocholate	+1·2	+1·8
5	Ascorbic acid	+1·9	+0·1
6	Sodium acetate	+0·2	+0·0
7	Citric acid	+0·1	+0·5
8	Acetic acid	+0·8	+3·8
9	Glycine	+3·1	+1·4
10	Gum Arabic	+2·9	+0·7
11	$(NH_4)_2 HPO_4$	+0·4	+0·3
12	$Na_2 HPO_4$	+0·8	+0·0
13	$KH_2 PO_4$	+2·1	+1·3
14	$MnSO_4$	+1·8	+0·7
15	NaCl	+0·3	+0·7
16	HCl	+0·2	+0·5
17	$CaCl_2$	+0·7	+0·2

From the above table it can be seen that out of the organic substances glycine, strychnine chloride and gum arabic are the best and sodium taurocholate is a good accelerator. From inorganic salts, $MnSO_4$ is the best accelerator. The salts used for buffer solutions accelerate or at any rate do not inhibit lipase action.

Comparison between Different Preparations of Lipase as regards their Activity

It is found that lipase slowly loses its activity after some time. Hence different preparations are made and their activities are studied at different intervals in order to see whether a stable lipase can be obtained.

Three samples of lipase are prepared by draining for two hours one with petroleum ether, the other with acetone and the third with the mixture of acetone and low boiling petroleum ether (B.P. 56° C.). All the samples are completely dried, powdered and sieved through 60-mesh sieve and used for the experiment. The hydrolysis of groundnut oil is carried out using each sample at different intervals of time. The results are given in Table VIII.

TABLE VIII

OIL : Groundnut Oil. Lipase : Castor lipase

Set No.	Time of ageing	Difference in terms of c.c. N/10 NaOH between the sample and the blank for		
		Petroleum dried	Acetone dried	Petroleum ether and acetone mixture dried sample
1	0 minutes	3·9	2·2	6·8
2	40 ,,	4·8	2·2	6·9
3	24 hours	5·2	2·3	6·9
4	2 days	6·1	2·3	7·0
5	4 ,,	7·8	2·3	7·2
6	6 ,,	6·5	2·2	7·2
7	8 ,,	5·6	2·2	7·2
8	12 ,,	5·3	2·2	7·1
9	15 ,,	4·9	2·2	7·1
10	23 ,,	4·7	2·1	7·1
11	45 ,,	4·3	2·1	7·0
12	55 ,,	3·9	2·1	7·0
13	65 ,,	3·5	2·1	7·0
14	75 ,,	3·0	2·1	6·9
15	85 ,,	2·8	2·1	6·9
16	95 ,,	2·6	2·1	6·9
17	105 ,,	2·1	2·0	6·9
18	115 ,,	1·9	2·0	6·7
19	125 ,,	1·5	2·0	6·7

From the above table, it can be seen that in all cases, the activity is maximum in 2–4 days. The activity of the lipase is the greatest in case of petroleum ether dried sample, slightly less in case of petroleum ether and acetone mixture dried sample and the least in case of acetone dried sample. But at the same time, the velocity of change in activity is the least in case of acetone dried sample.

Hence acetone dried sample can be used for further experiments as its activity remains fairly constant even though its activity is less.

SUMMARY

A general study of the lipase obtained from different oil seeds is made.

1. The optimum pH changes to a very small extent with the nature of the substrate and it does not change much with the nature of the buffer.

Castor and groundnut lipases are the best active; groundnut oil is the best substrate and sodium acetate-acetic acid is the best buffer.

2. The optimum buffer concentration is 1–2 c.c.; the optimum substrate concentration is 1–2 c.c. and the optimum temperature is 35–37° C. depending upon the nature of the lipase.

3. The percentage hydrolysis of the oil goes on increasing with the enzyme concentration.

4. Organic substances like glycine, gum arabic and sodium taurocholate and inorganic salt $MnSO_4$ accelerate the activity of the lipase.

5. The acetone dried sample of the lipase keeps its activity fairly constant for an appreciably long time.

References

1. Ramakrishnan, C. V. and Nevgi, G. V. .. *Jour. Ind. Chem. Soc.*. 1950, **27**, 255–62.
2. Longnecker, H. E. and Haley, D. E. .. *Jour. Am. Chem. Soc.*, 1935, **57**, 2019.
3. McIlvaine .. *J. Biol. Chem.*, 1921, **49**, 183.
4. Walpole .. *Bio. Chem. Jour.*, 1914, **105**, 257.

THE ROLE OF TYPE OF SEED IN THE CULTURE OF POTATO

Relative Vigour of Apical, Vertical and Basal Sets

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(Communicated by Dr. B. Viswa Nath, F.A.Sc.)

Views differ on the relative vigour of apical and basal sets. Sanborn (1891), Close and White (1909) and Ellis (1942) conclude that bud-end sets are superior to stem-end pieces. Atwood (1891), Speer (1891), Werner (1919), Stuart *et al.* (1927), Westover (1930) and Lombard and Stuart (1936), on the other hand, report to have found no difference in yield from stem, middle and seed-end pieces. There is yet another set of experiments which prove middle and basal pieces to be superior to apical sets. Thus Halsted (1897, 1899) obtained larger yields from the middle sets with yields from the basal sets out yielding the apical sets. Similarly Appleman (1918) and Myers (1934) report large yields from sets taken from the basal end.

The problem of relative value of different sets thus required to be studied and particularly when it is seen that the potato grower in India practises a great laxity in the preparation of sets. Little regard is paid to the position and relative vigour of different eyes in the seed tuber and the only care taken is to include in each piece at least one eye.

The present work was, therefore, undertaken to study the vigour of apical, vertical and basal sets in order to determine a suitable plan of making sets.

Methods

In India certified potato seed is not available (Ramanujam, 1947). It is, therefore, extremely important that the initial seed material for experiment should be chosen with great care.

In the present investigation seed tubers (var. Darjeeling red round) were obtained through the Directorate of Agriculture, West Bengal. A rigorous selection was made to obtain potatoes of similar size, shape and other tuber characters. All diseased and damaged tubers were discarded and since planting was done with cut pieces those that showed, on cutting, disease symptoms were further rejected.

Apical and basal pieces were prepared by cutting the seed tubers transversely. The vertical pieces were obtained by giving a cut extending from the bud end towards the stem-end side so that each piece included both the type of eyes. Weight of each set was kept as $20 \pm 1 \cdot 0$ gm. but due to unequal distribution of eyes which are more towards the 'rose' end, it proved difficult in spite of much care to maintain a uniform number of eyes in all the three types of pieces. Prepared sets were sown in cement concrete pots of $12 \times 15''$ size containing field soil and $\frac{3}{4}$ lb. cowdung manure per pot. There were 4 replications and 15 pots under each treatment. Observations were made on the germination behaviour, growth and yield characters.

Results

Germination behaviour.—Data are shown in Table I. All the type of sets send out sprouts on the 6th day after sowing but number of germinations is maximum for the apical sets. A similar trend is maintained throughout the period of germination and bud-end pieces record a higher percentage of germination than either the vertical or the basal. This indicates that these are relatively more vigorous. Duration of germination is also less for the apical sets and the whole lot completes the process by 12 days as against 16 days for the vertical and basal sets. Thus, in speed as well as duration of germination, the bud-end set proves superior. Next in order is the vertical set. As regards capacity of germination, apical and vertical sets give 100 per cent. emergence and the basal set 96·66 per cent. which shows that besides slow germination, the percentage is also low in case of basal pieces.

TABLE I. *Speed, duration and capacity of germination of apical, vertical and basal-end sets*

Number of pots per treatment, 60

Type of set	No. of sprout come-up on days after sowing								Germination per cent.
	4	6	8	10	12	14	16	18	
Apical	19	32	48	60	60	60	60		100·00
Vertical	16	25	38	47	58	60	60		100·00
Basal	8	20	28	44	52	58	58		96·66

Growth studies.—Advantage gained during germination is maintained by the apical set till late in the life period. It is, however, observed that the differences which are more in the beginning become relatively less later

on (Table II). This is particularly true when compared with vertical sets. Thus, at 45 days age the growth of top for apical, vertical and basal piece is 41·20, 37·95 and 30·87 gm. respectively while at 90 days age the relative values are 127·60, 127·75 and 120·02 gm. per hill. Tuber growth follows a trend similar to that exhibited by the top.

TABLE II. *Fresh weight of top and tubers per hill in grams at successive stages of growth*

Type of set	Age in days				
	45	60	75	90	105
		TOP			
Apical ..	41·20	64·97	114·25	127·60	..
Vertical ..	37·95	62·72	113·62	127·75	..
Basal ..	30·87	59·65	108·87	120·02	..
		TUBER			
Apical ..	8·65	46·80	99·80	129·60	173·82
Vertical ..	7·97	41·60	103·60	129·40	173·12
Basal ..	6·60	38·55	87·87	127·00	164·42

Yield and grade.—Data are presented in Table III. Highest yield is recorded from the apical set which shows an increase of 3·77 gm. over vertical and 13·62 over stem-end set. Critical difference is 14·08 and increase over the stem-end set narrowly misses the level of significance.

TABLE III. *Average yield, number and size of potato obtained from plants raised from apical, vertical and basal-end sets*

Mean of 40 hills

Type of set	Yield per hill in gm.	Number per hill	Average size in gm.
Apical ..	173·52	11·35	15·31
Vertical ..	169·75	10·50	16·21
Basal ..	159·90	8·57	18·69
C.D. at 5% ..	14·08	1·31	1·65

Number of tubers per hill is also more in case of apical pieces. Apical set gives 11·35, vertical produces 10·50 and the basal only 8·57 tubers per hill.

As regards average size of potatoes a better grade of produce is obtained from the basal set. An overall comparison of the three type of sets shows that apical set behaves significantly different from the basal set but differences between apical and vertical are small and prove insignificant.

Discussion

Apical sets have given better germination, rapid growth and a higher yield. The number of tubers is also more. Average size of tubers is, however, relatively small.

A study of orientation of buds on the potato tuber shows that buds are far apart at the stem end and get closer together at the terminal end. Apical sets, thus, have more eyes and apart from higher frequency, buds on the apical end are reported to be more vigorous also (Bushnell, 1929). A better germination behaviour is, therefore, obvious.

Further, the apical end shows dominance over the basal end. It inhibits the growth of basal buds and keeps them in suppression. Accordingly when the whole tuber is cut and the apical and basal sets are sown, the buds on the apical set which are vigorous and already in a sprouting condition shoot out soon while the buds on the basal set which were till late (prior to cutting) in a dormant form and under suppression, lag behind. And on germination, the large sprout of the apical piece gains the advantage of having grown to and through the stage which the smaller sprout of the basal piece has yet to reach. Better germination and rapid initial growth, therefore, contribute towards a relatively higher yield from apical sets. Stewart (1922), likewise, concludes that subsequent conditions being equal, plants making a rapid growth early in the season outyield plants which grow more slowly.

Further experiments in this connection are in progress at this research station.

Influence on tuber count and average size of tubers is explicable on the basis of number of sprouts per hill. Average number of sprouts per hill obtained in different cases is given below:

Apical set $=2\cdot90$; Vertical set $=2\cdot67$ and Basal set $=2\cdot05$.

Larger number of sprouts or stalks (which actually are true plants) naturally produce more tubers but an increase in the number of plants causes a corresponding increase in the intensity of competition within the hill and the average size of potato consequently falls down.

Summary

Rapid germination, better growth and higher yield is obtained from apical sets. Vertical sets prove slightly inferior; the difference, however, is too small to claim much significance. Basal sets show poor performance and are likely to cause ' missing hills ' in the field.

It is concluded that when planting is intended with cut pieces, sets should be prepared by cutting the seed tubers longitudinally, from the bud-end towards the stem-end, in order to include in each piece a part of the bud-end also.

Acknowledgments

Grateful thanks are due to Dr. B. Viswa Nath and Principal J. C. Luthra for their valuable suggestions.

Literature Cited

1. Appleman, C. O. .. "Physiological basis for the preparation of potatoes for seed," *Maryland Agr. Exp. Sta. Bul.*, 1918, **212**, 79–102.
2. Atwood, W. B. .. .. "Potato tests," *Va. Agr. Exp. Sta. Bul.*, 1891, **8**, 23.
3. Bushnell, J. .. .. "The normal multiple sprouting of seed potatoes," *Ohio Agr. Exp. Sta. Bul.*, 1929, **430**, 1–27.
4. Close, C. P. and White, T. H. .. "Irish Potato investigations," *Md. Agr. Exp. Sta. Bul.*, 1909, **132**, 151–73.
5. Ellis, N. K. .. .. "A comparison of stands and yields from seed pieces cut from the apical and stem ends of Irish Cobbler, Russet Rural and Chippewa potatoes," *Proc. Amer. Soc. Hort. Sci.*, 1942, **40**, 516–18.
6. Halsted, B. D. .. .. "Further experiments with potatoes," *N. J. Agr. Exp. Sta. Ann. Rept.* (1896), 1897, **17**, 315–18.
7. ——— .. .. "Potato experiments after turnips," *Ibid.*, (1898), 1899, **19**, 307–09.
8. Lombard, P. M. and Stuart, W. .. "Relative vigour of apical and basal seed piece of potato," *Amer. Pot. Jour.*, 1936, **13**, 124–30.
9. Myers, C. H. .. .. "Improving the potato crop by selection," *N. Y. State Col. Agr. Cornell Reading Courses*, 1924, **3**, 229–48.
10. Ramanujam, S. .. .. "Potato seed certification for India," *Ind. Farming*, 1947, **8**, 616–22.
11. Sanborn, J. W. .. .. "Potato trials," *Utah,. Agr. Exp. Sta. Bul.*, 1891, **5**, 1–22.
12. Speer, R. P. .. .. "Experiments with potatoes," *Iowa Agr. Exp. Sta. Bul.*, 1891, **12**, 507–52.
13. Stewart, F. C. .. .. "Potato seed experiments. Whole small tubers *vs.* pieces of large tubers of the same plant," *New York Agr. Exp. Sta. Bul.*, 1922, **491**, 1–30.
14. Stuart, W. *et al.* .. .. "Source, character and treatment of potato sets," *U.S. Dept. Agric. Tech. Bul.*, 1927, **5**, 1–33.
15. Werner, H. O. .. .. "Potato experiments 1917 and 1918," *North Dakota Agr. Exp. Sta. Bul.*, 1919, **129**, 1–22.
16. Westover, K. C. .. "The relative vigour of the bud and stem ends of the same potato seed tuber," *Proc. Ann. Meet. Potato Assoc. Amer.*, 1930, **16**, 101–05.

STUDIES ON PLANT GROWTH IN RELATION TO DIFFERENT SOILS AND MANURES

I. Nitrification Studies of Different Soil Types with Different Manures

By Prof. Shri Ranjan, F.A.Sc. and M. D. Kothiyal

Received May 19, 1950

Introduction

Nitrogen is an index of soil fertility, it being the most important limiting factor of Indian soils. It is already known that the nitrogen in manure is made available to the plant through micro-organic activities of the soil which are governed by a number of factors of which the following are important:—(*a*) Temperature, (*b*) moisture, (*c*) Physico-chemical properties of soils, (*d*) nature and amount of oxidising materials present, (*e*) microflora. The last three factors are intimately linked up with each other and find their full expression in a "soil type".

The present study is to find out the effect of different "soil types" upon the nitrifying power, keeping the rest of the factors constant and supplying equal amounts of nitrogen through different manures.

Material and Method

Soil Type.—After an analysis of a number of soils from different localities of Allahabad District, four soils with wide textural differences were selected which had normal chemical components for plant growth. They were:

(*a*) *Silty clay soil.*—From Sulem Sarai.

(*b*) *River bed soil* (*Silt loam*).—From Ganges river bed near Daragunj.

(*c*) *Loamy soil.*—From Botany Department Gardens.

(*d*) *Sandy soil.*—From Botany Department Farm, near Beli Village, Allahabad.

Approximately 10 maunds of soil from a uniform levelled part under cropping system, but not manured, was dug from 9″ depth and passed through 3 mm. sieve. Earthenware pots, 12″ × 12″, were filled with 30 lb. of air-dry soil for nitrification studies. A similar study in bottles at constant temperature was also started.

Manure

Four commonly used manures were selected. They were:

(*a*) *Farmyard manure.*—It was pure cow dung with little cattle shed refuge. It was composted in small pots or heaps with occasional stirring and used after 3–4 months.

(*b*) *Green manure.*—Sunn hemp was obtained from Agricultural Institute, Naini, which was grown for green manuring the fields. Sunn hemp plants were uprooted just before flowering and cut into pieces of 1 cm. Representative samples were analysed in triplicate for total nitrogen and moisture.

(*c*) *Leaf compost.*—Non-leguminous leaves were collected from leaf fall and other causes and composted in a pit for 4–5 months. Representative samples of these were also analysed.

(*d*) *Inorganic.*—A mixture of ammonium sulphate, potassium sulphate, sodium nitrate, calcium phosphate was used so as to make it equivalent to, total nitrogen, total phosphorus and total potassium contents of the farmyard manure used. Ammonium sulphate and sodium nitrate were used in the proportion so that 50% of nitrogen was contributed by each of these two chemicals.

Soils in bottles were kept at a fixed temperature of 32° C. and pots were exposed to sun.

Pot study for nitrification was started on Aug. 23, 1948, and the bottles study from January 1, 1949.

Periodic study of total available nitrogen was done (consisting of nitrate nitrogen, and ammoniacal nitrogen) after 50 days, 100 days and 135 days in pots and after 35 days, 60 days and 95 days in bottles.

Total nitrogen was determined by Gunning and Hibbards modification of Kjeldahl's method, ammonical nitrogen by Mc. Leans and Robinson's Process and nitrate nitrogen of the same sample by reducing remaining nitrates with Devarda's alloy and absorbing ammonia produced in N/10 sulphuric acid and estimating by Duboseque colorimeter.

Observations

Original values of soil

	Total nitrogen	Available nitrogen
Clay soil ..	0·045	0·004
Garden soil ..	0·080	0·004
Kachchar soil ..	0·085	0·006
Sandy soil ..	0·050	0·002

The above values are in grams per cent. on oven-dry basis.

For Pot Study (*started on Aug.* 23, 1948)

Three observations were taken, namely after 50 days, 100 days and 135 days. The observations are as follows:—

	After 50 days	After 100 days	After 135 days
	(Garden soil)		
F. Y. Manure ..	8·34	3·02	6·30
Green Manure ..	3·42	2·21	3·40
Leaf Compost ..	2·32	2·79	2·28
Inorganic ..	3·89	3·40	4·08
	(Botany Farm soil)		
F. Y. Manure ..	4·72	2·69	3·38
Green Manure ..	4·25	2·01	4·15
Leaf Compost ..	3·37	3·05	2·71
Inorganic ..	5·32	2·61	5·95
	(Kachhar soil)		
F. Y. Manure ..	4·25	6·03	8·60
Green Manure ..	4·73	6·11	7·11
Leaf Compost ..	6·35	7·13	6·75
Inorganic ..	7·03	11·60	4·55
	(Silty clay soil)		
F. Y. Manure ..	2·51	3·58	4·62
Green Manure ..	5·23	3·92	4·25
Leaf Compost ..	6·15	3·43	6·88
Inorganic ..	4·50	4·63	3·89

For Bottle Study (*started on Jan.* 1, 1949)

Three observations were taken, namely after 35 days, 60 days and 90 days. The observations are as follows:—

	After 35 days	After 60 days	After 95 day
	(Garden soil)		
F. Y. Manure :	6·99	9·48	4·72
Green Manure ..	7·88	8·11	8·06
Leaf Compost ..	9·67	9·70	15·68
Inorganic ..	12·04	10·68	12·73
	(Botany Farm soil)		
F. Y. Manure ..	7·79	8·97	4·38
Green Manure ..	5·37	6·15	5·79
Leaf Compost ..	6·31	9·62	10·25
Inorganic ..	13·05	15·62	10·13
	(Kachhar soil)		
F. Y. Manure ..	15·09	17·25	16·08
Green Manure ..	12·28	14·05	13·15
Leaf Compost ..	8·75	20·89	18·52
Inorganic ..	9·47	12·96	13·95
	(Silty clay soil)		
F. Y. Manure ..	18·59	17·02	18·80
Green Manure ..	8·86	15·69	23·08
Leaf Compost ..	16·59	16·09	20·89
Inorganic ..	18·02	14·86	20·74

All the above values are in mgm. per cent. on oven-dry basis.

Discussion

Nitrification is an aerobic process depending upon the amount of oxygen present in the soil. It is also a bacterial and photo-chemical process and as such depends upon the surface exposed for interaction. Upon the texture of the soil depends both these characters and the present observations reveal some interesting results. The observations are discussed one by one with different treatments of manures.

Some important points that have been taken into consideration are:—

In bottle study, due to water supply, there has been no case of leaching. Pot soils that were kept at the field conditions open to sun and rain were leached. Moreover the pot culture experiments were conducted during rainy season (started in Aug.) and as such leaching seems to have been the only reason for loss of nitrogen, especially in sandy soils that have the greatest pore space. It will be clear from the following discussion.

In Case of F. Y. Manure.—In bottle study (Fig. 2) after a gradual rise, reaching its maximum there is gradual fall. The fall is maximum in sandy soil, lesser in loamy soil, least in silty and a rise in clay soil. The slow rise and fall in clay soil is due to the least oxygen supply in that soil.

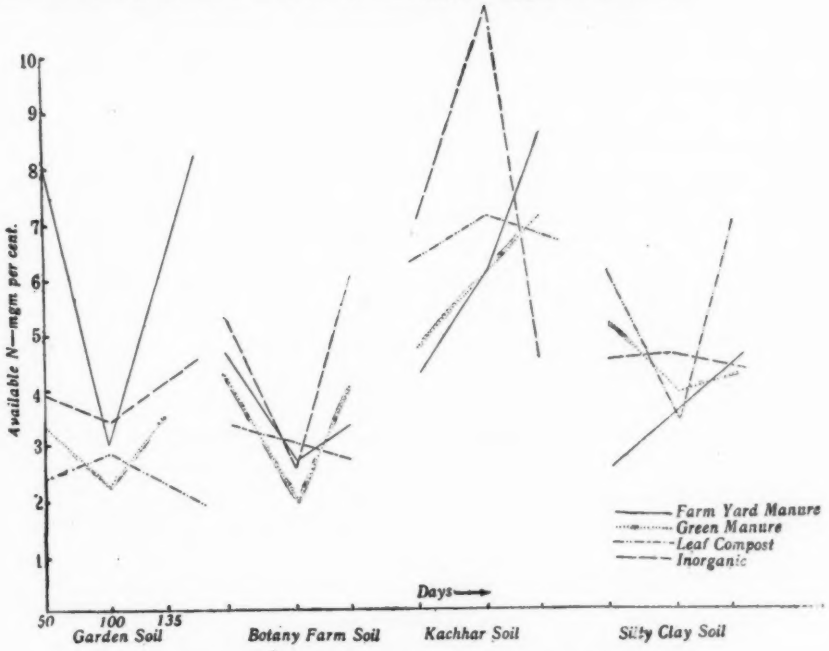

Fig. 1. *Nitrification studies in pots* started on Aug. 23, 1948

In pot study (Fig. 1) there is a sudden fall due to leaching and then a slow rise in nitrogen content. The fall is most marked in sandy soil, and practically no fall in silty soil, and a rise in clay soil. It can be well explained from the texture-sandy soils allowing maximum leaching and clayey the least due to greater non-capillarity pore space.

Leaf Compost—Records a rise even after 30 days in farm soil in bottle study (Fig. 2), where aeration was better, later so (after 60 days) in garden soil. Kachhar soil that provide the best medium, for aeration and water reaction, as well as a greater surface for reaction shows quite a rapid rise. Fixation of nitrogen in leaf compost starts only after decomposition of leaf material and C/N ratio coming to 10. In clayey soil, the reaction is delayed due to lesser permeability of air. In pot study (Fig. 1) after due decomposition the fall is due to washing away of fixed nitrogen. It is checked in kachhar and clayey soils.

Green Manure.—Sanai has got a low C/N ratio (7·6) and as such after some time amount of nitrogen falls (the fall starts quite early in pot study which is open to sun and rains, thus hastening the reactions). The rise takes place only after 100 days in almost all soils (more rapidly in Farm soil) (see Fig. 1). In bottle study (Fig. 2) values are more or less constant in farm

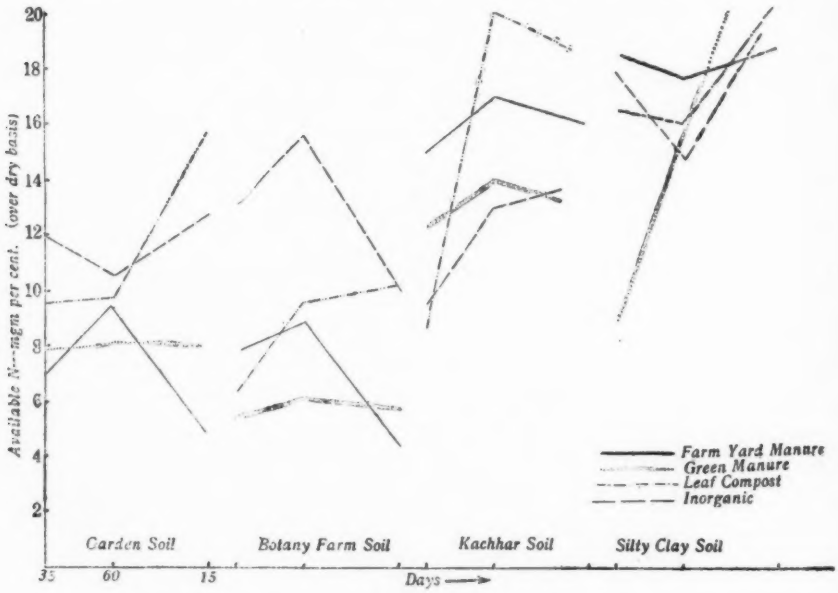

FIG. 2. *Nitrification studies in bottles* started in Jan. 1, 1949

soil and garden soil. The rise in clay soil and kachhar soil in pot study seems to be due to some irregularity.

Inorganic Salts.—The washing of these salts starts quite early in sandy and later so in garden soil, after 100 days in kachhar and very slowly in clay soil, that have greatest capacity of retention. Rise afterwards may be due to natural nitrogen fixation.

In bottle study (Fig. 2) fall takes place first in farm soil, later so in others.

Due to high percentage of carbon in kachhar and garden soils, some fixation has no doubt taken place even by the addition of inorganic salts, considering due disturbances in C/N ratio.

Conclusion

In the process of nitrification both air and moisture play a very important role and it is only after a proper adjustment of these that a good fixation can be obtained. Sandy soils that provide a good aeration due to large non-capillary pore space, should be regarded as best for nitrification, yet they allow greatest leaching and no retention of water, thereby loosing all their nitrogen contents very easily. Finer soils that provide lesser amount of air, but proportionate retention of water in them are best fitted for nitrogen fixation. They also provide greater surface for reaction. More finer soils are impervious to oxygen supply and as such are not well suited for rapid nitrogen fixation. These soils are slow in action.

Only a medium is needed for a proper and rapid fixation of nitrogen. All these different soil conditions can be improved very easily with the application of different manures, *e.g.*, green manure in clay soils, and carbonaceous matter in sandy ones.

Note.—Due to the untimely death of the second author the work could not be completed and a number of observations regarding pH, mechanical analysis of the soil, carbon contents at different stages, potash, phosphate and total nitrogen value and separate ammoniacal nitrogen and nitrate nitrogen could not be obtained. However, some of the salient points, that could be easily drawn out and cannot be neglected, are given in this paper, which also gives testimony of the single-minded devotion, of the late Mr. Kothiyal, to research. The senior author thanks Mr. Atma Prakash for shifting the data and bringing it up to a form suitable for publication.

STUDIES ON PLANT GROWTH IN RELATION TO DIFFERENT SOILS AND MANURES

II. Manurial Properties of Mango Seed Kernel*

By Prof. Shri Ranjan, F.A.Sc., M. D. Kothiyal and Atma Prakash

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The economic dependence of the country brings out one fact namely that the so-called waste products should be harnessed. One such material, mango kernel is thrown away as waste. It is estimated that about a million tons of such kernel is so wasted. An interesting paper by Kehar Chandra[1] has shown that when properly treated such kernel can be profitably used as food for human consumption, if mixed in suitable amount with cereals. However, prejudices die hard, and such mixtures do not prove quite palatable for human taste. It was, therefore, decided to explore further the possibilities of using this as a manure for raising cereal crops.

Mango seed kernel contains approximately 0·961 to 1·4% of total nitrogen and approximately 8·50% of crude protein. The value of total nitrogen will differ within the above range in different samples. On composting the kernel for two months the nitrogen content rises to 3·17 gm. per cent.

Powdered mango seed kernel and mango seed kernel compost was used in statistically laid out pot culture experiments in the doses of 20, 40, 80 and 100 lbs. of nitrogen equivalent per acre, taking 20 lbs. of soil in each pot and taking it to be 0·000,001 part of an acre of land. Farm yard manure, green manure, non-leguminous leaf-compost, wheat flour and no-manure were included in the experiment for comparison. Wheat contains approximately the same amount of protein (9·65%) as mango seed kernel (8·50%) and so it was interesting to investigate the influence of proteinous matter on soil fertility.

Three crops were raised in these pots, wheat followed by paddy and maize and that followed by wheat again. Nitrification studies in bottles in an incubator at 32° C. and optimium moisture were done. Fortnightly

* The work was undertaken at the suggestion of the Senior author by the late Mr. M. D. Kothiyal. Due to his untimely death the work could not be completed by him. The fragmentary data left by him was collected, seived, and added by the third author.

[1] *Curr. Sci.*, Feb. 1946, page 48.

growth observations of tillers, height, leaf number and yield were recorded on individual plants.

Data for crop response and yield show that 20 gms. per pot or 40 lbs. of nitrogen equivalent of mango kernel powder as well as compost gives as good a plant response as 100 lbs. of nitrogen equivalent of farm yard manure. The chemical analysis after the harvest shows that it leaves as good a residual effect as the farm yard manure. The use of composted mango kernel gives high significant differences in yield and proves superior to farm yard manure. Higher doses of kernel have a depressing effect on the crop yield due to high C/N ratio. The crop response and yield by using wheat flour in 100 lbs. of nitrogen equivalent as manure was most remarkable. It gave 100% increase in yield.

Nitrification studies show that 20 gms. mango kernel per pot releases as much of nitrates as the 100 lbs. nitrogen equivalent of farm yard manure. Wheat flour does not show any superiority in its nitrifying power as compared to mango kernel and farm yard manure but still exercised remarkably beneficial influence on crop growth and yield.

A CONTRIBUTION TO THE EMBRYOLOGY OF *DROSERA INDICA*, L.

By S. G. Narasimhachar, M.Sc.

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Received July 20, 1950

(Communicated by Dr. L. S. Dorasami, F.A.Sc.)

Introduction

Earlier literature on the embryological features of Droseraceæ has been reviewed by me elsewhere (Narasimhachar, 1949). Recently Venkatasubban* (1950) has described in detail the development of the male gametophyte in the *Droseraceæ*. The present paper deals with the embryology of *Drosera indica*. It is a small erect herb about 4 to 6 inches in height and flowers during November and December. The material was collected at Bannerghatta about 12 miles from Bangalore and fixed in Formalin-Acetic-Alcohol and Allen's modified Bouin's fluid. After following the customary methods of preparation for study, sections were cut at a thickness of 6 to 8 microns and stained in Heidenhain's Iron-alum-Hæmotoxylin.

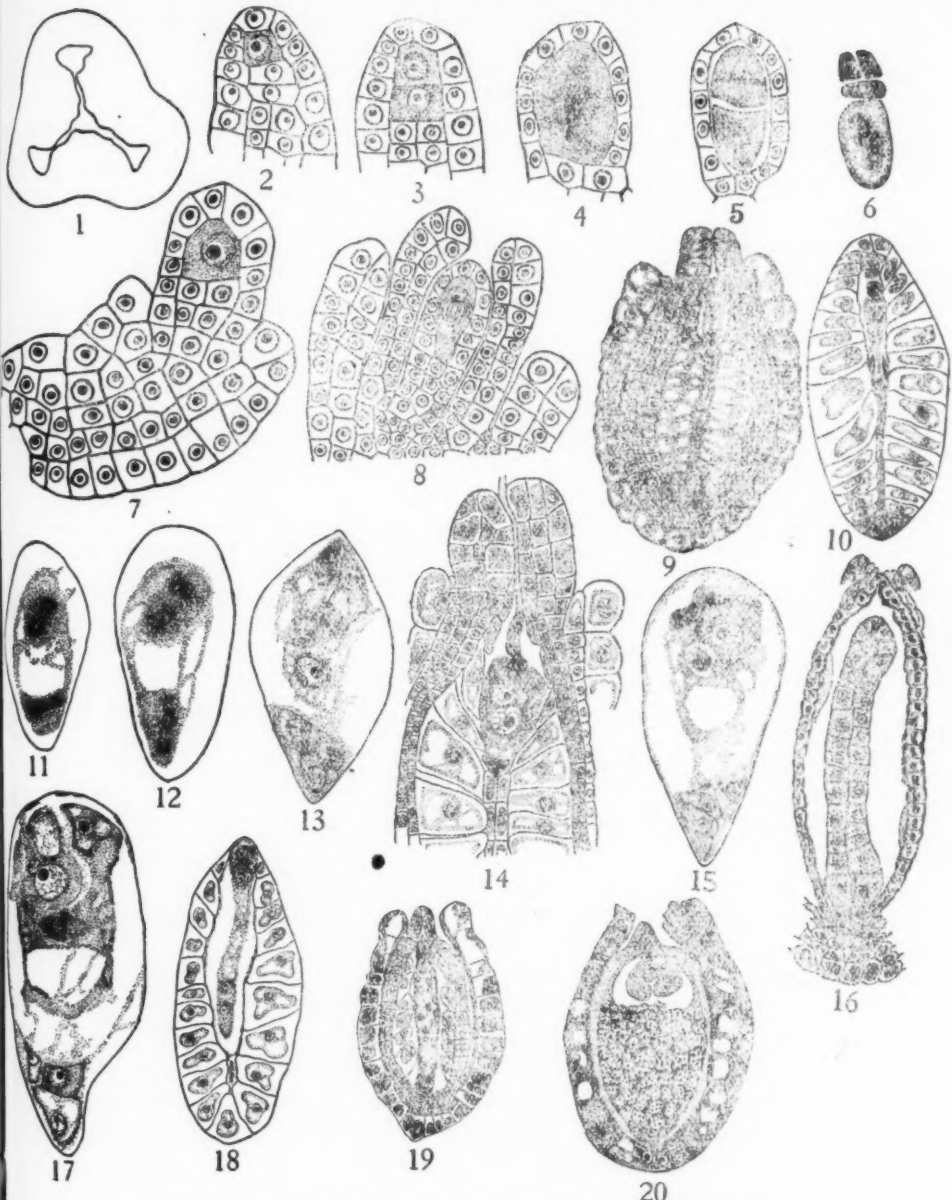
Organogeny

The different floral parts take their origin in acropetal succession, *viz.*, sepals, petals, stamens, and finally carpels.

Megasporogenesis and Development of the Female Gametophyte

The ovary is superior, unilocular with a number of anatropous bitegmic ovules attached to the parietal placenta (Fig. 1). The ovule takes its origin as a small nucellar primordium on the placenta. Next the two integuments are differentiated. Of the two integuments, the inner integument is the first to develop (Figs. 7 and 8). Later it is followed by the outer integument. The micropyle is formed only by the inner integument (Figs. 9 & 14). Both the integuments are two cells thick, but the inner however becomes a few more layered at the micropylar region (Fig. 14). The inner layer of the inner integument during the stages of endosperm development becomes regularly organised (Fig. 19). At a later stage the cells of this layer lose their contents gradually thus probably serving a nutritive role (Figs. 20 and 46, 47). At

* When this paper was in the Press, another paper has appeared on the Droseraceæ by Venkatasubban (1950).



FIGS. 1–20. Fig. 1. Transverse section of an young ovary showing the placenta, × 400. Fig. 2. Hypodermal archesporium, × 1,800. Fig. 3. Double archesporium, × 1,800. Fig. 4. Metaphase in the megaspore mother cell, × 1,800. Fig. 5. Division of the diad

to form the T-shaped tetrad, × 1,800. Fig. 6. Degeneration of the upper three megaspores with the functional megaspore wherein the nucleus is dividing, × 1,800. Fig. 7. Megaspore mother cell and origin of the inner integument, × 1,800. Fig. 8. Late telophase in the megaspore mother cell to form the diad and the inner and outer integuments advanced in development, × 1,800. Fig. 9. Longitudinal section of ovule showing the fully organized embryo-sac with the egg apparatus, fusion nucleus, antipodals, and the outer and inner integuments, with the transversely elongated nucellar epidermis and linearly elongated nucellar cells, × 480. Fig. 10. An embryo-sac with degenerated egg and synergids, healthy fusion nucleus and early degeneration of antipodals, × 800. Figs. 11 & 12. Two- and four-nucleate embryo-sacs in division to form the four- and eight- nucleate embryo-sacs respectively, × 1,800. Fig. 13. Early embryo-sac showing the egg apparatus and antipodal cells. One of the polars is just below the egg while the other one is moving up, × 1,800. Fig. 14. Entry of the pollen tube with the two male cells into the embryo-sac, × 1,800. Fig. 15. A stage in double fertillzation wherein the male nuclei are in contact with the egg and fusion nucleus. The antipodals are still persisting, × 1,800. Fig. 16. Differentiation of the cellular endosperm into three regions, *viz.*, the micropylar region wherein the nuclei are dividing in the small endosperm cells, the middle region consisting of big cells with reserve material and chalazal region with dividing cells and the gradual disorganization of the inner layer of the inner integument, × 480. Fig. 17. First division of the endosperm nucleus. The antipodals are still persisting, × 1,800. Fig. 18. Division of the fertilized egg when there are eight endosperm nuclei and disorganization of the linear nucellar cells, × 480. Fig. 19. 5-Celled embryo and the beginning of the disorganization of the nucellar epidermis and the deposition of tannin in the cells of the outer integument, × 200. Fig. 20. Longitudinal section of a mature seed showing the dicotyledonous embryo and endosperm cells filled with rich contents and depletion of the contents of the inner layer of the inner integument, × 200.

the same time the outer cells of the outer integument become large and conspicuous when the embryo-sac is mature and after fertilisation acquire dense brownish contents of the nature of tannin (Figs. 19 and 20). The outer layer of the inner integument and inner layer of the outer integument become crushed in the mature seed.

The nucellus is small and the arechesporium is hypodermal in origin (Figs. 2 and 7). Occasionally two archesporial cells are met with (Fig. 3), though only one develops further. It functions directly as the megaspore mother cell and as development proceeds further crushes the nucellar cells surrounding it. It undergoes the usual meiotic divisions (Figs. 4, 8 and 5) and forms a T-shaped tetrad of megaspores (Fig. 6) of which the lowermost develops into the eight-nucleate embryosac (Figs. 11, 12, 13 and 9) according to the Polygonum type (Maheshwari, 1948). The mature embryo-sac is broad in the centre and pointed at either end. The synergids are triangular and slightly beaked laterally (Fig. 13) with a narrow anterior end. A vacuole is present beneath the nucleus of each synergid. The pear-shaped egg is longer and is situated between the synergids. The polar nuclei meet below the egg and fuse to form a large secondary nucleus prior to fertilisation. The antipodals are organised as definite cells and may persist until the first division of the endosperm nucleus (Fig. 17). The mature embryo-sac

is found only in the upper region of the nucellus and is surrounded by the nucellar epidermis; the cells of this layer below the embryo-sac become transversely elongated and enclose vacuolated cytoplasm. The remaining cells of the nucellus in the central region become vertically elongated and function as a conducting strand connecting the antipodal end of the embryo-sac with the chalazal region of the ovule (Fig. 9). At a later stage, the entire nucellar epidermis gradually gets crushed.

The pollen tube enters through the micropyle either between the synergids or destroying one of them (Figs. 14 and 15). Syngamy and triple fusion take place as normal processes during fertilization. Usually syngamy is slightly belated.

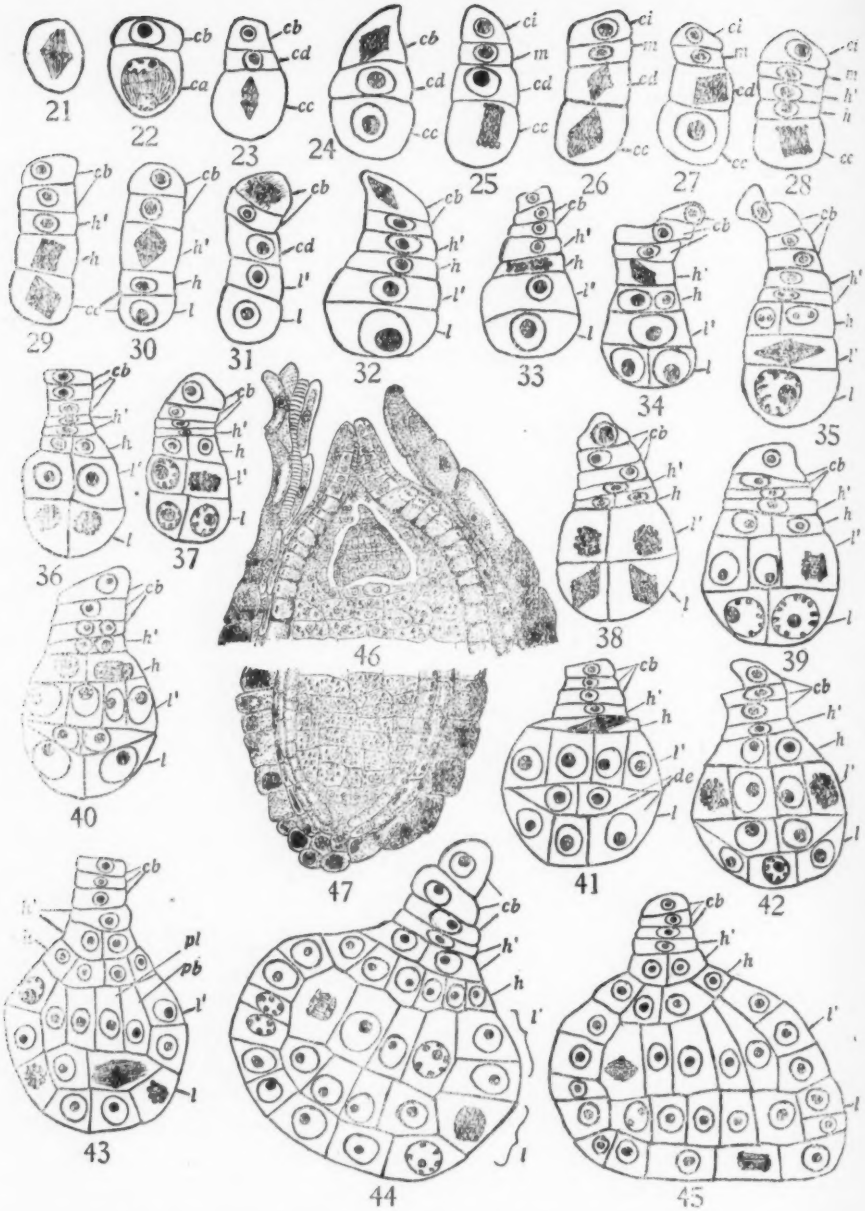
Endosperm

The primary endosperm nucleus divides much earlier than the fertilized egg (Fig. 17). After many repeated free nuclear divisions, they become densely aggregated at the micropylar region around the embryo and at the chalazal end, as in *Drosera Burmanni* (Narasimhachar, 1949). At a later stage wall formation sets in around these nuclei and centripetal growth is initiated from the micropylar end. After cytokinesis the endosperm cells situated at the top and the base show further divisions (Fig. 16), the central cells remaining as such for some time. Usually the endosperm cells surrounding the embryo are small in size; but those at the chalazal end are larger in size. The centrally situated cells are filled with dense contents and proteid crystals (Figs. 16, 20 and 46, 47).

Embryo

The fertilized egg divides transversely (Fig. 21) forming two cells *ca* and *cb*. The upper cell *cb* divides transversely producing *m* and *ci* (Figs. 25 to 28). Next *ci* by one more transverse division produces two more cells (Figs. 29 to 45). Thus the three cells formed from *cb* contribute towards the length of the short suspensor.

The lower cell *ca* by further transverse divisions produces cells *l*, *l'*, *h*, and *h'*. These divisions may take place one after another or almost simultaneously. Now in cells *l* and *l'* vertical walls are laid down at right angles to the first resulting in the octant stage (Figs. 34 to 41). Next periclinal walls are laid down in these two tiers (Figs. 37 to 41), thus delimiting the outer dermatogen from a group of inner cells. Meanwhile cell *h'* divides transversely (Fig. 34) producing two cells (Figs. 35 to 45). Of these the upper cell adds to the length of the suspensor. The lower cell undergoes a vertical division (Figs. 40 to 45) and takes part in the formation of the embryo.



FIGS. 21–47. Figs. 21 to 45. Stages in embryogeny. All, × 1,800. Fig. 46. Late embryo with the big outer cells of the outer integument and complete depletion of the cell contents of the

inner cells of inner integument with the vascular strand traversing the outer integument, × 480.
Fig. 47. Lower portion of a seed showing the endosperm cells with rich contents, × 480.

ca., =Terminal cell ; *h* & *h.'* =Derivatives of *cd.* ;
cb., =Basal cell ; *m* & *ci.*, =Cells derived from *cb.* ;
cc. & *cd.*, =Cells derived from *ca* ; *de.*, =Dermatogen ;
l & *l'.*, =Derivatives of *cc.* ; *pe.*, =Periblem ; *pl.*, =Plerome.

After the delimitation of the dermatogen, the inner cells in tiers *l* and *l'* by further periclinal divisions form the future periblem and plerome (Figs. 43 to 45). Next periclinal walls are also laid down in tier *h* resulting in a semicircular group of cells. Of these, the inner cells complete the periblem in the upper part of the embryo. The lower cells of tier *h'* which has undergone a vertical division completes the dermatogen in the upper portion of the embryo (Fig. 45). A similar course of development with certain minor differences has been described in detail by Souèges (1936) for *Drosera rotundifolia*. The mature embryo is dicotyledonous and the seed is filled with rich contents.

Grateful acknowledgements are made to Sri. K. H. Srinivasan, Director of Agricultural Research, and Dr. L. S. Dorasami, Economic Botanist, for kind encouragement and criticism and Sri. K. Subramanyam for helpful suggestions.

Summary

1. The primary archesporial cell directly functions as the megaspore mother cell. Megasporogenesis proceeds normally and the embryo-sac conforms to the Polygonum type. Antipodals are formed as definite cells and persist for some time. At the mature embryo-sac stage the nucellus becomes characteristically differentiated.

2. Endosperm is free nuclear to start with. Later it becomes cellular.

3. The development of the embryo has been followed in detail and broadly corresponds to the Solanad type of Johansen (1945).

Literature

Johansen, D. A. .. "A critical survey of the present status of plant embryology," *Bot. Rev.*, 1945, 87–107.

Maheshwari, P. .. "The Angiosperm embryo-sac," *ibid.*, 1948, **14**, 1–56.

Narasimhachar, S. G. .. "A contribution to the embryology of *Drosera Burmanni Vahl.*," *Proc. Ind. Acad. Sci.*, 1949, **29**, B, 98–104.

Souèges, R. .. "Embryogénie des Droseracées. Development de l'embryon chez le *Drosera rotundifolia*," *C. R. Acad. Sci.*, Paris, 1936.

Venkatasubban, K. R. .. "Studies in the *Droseraceæ*. 1. The cytology of *D. indica.* Linn., *D. Burmanni* Vahl. and *D. peltata* Sm. with special reference to Pollen mitoses," *Proc. Ind. Acad. Sci.*, 1950, **31B**, 308–30.

———— .. "Studies in the *Droseraceæ*. II. A contribution to the embryology of three species of *Drosera*. *Proc. Ind. Acad. Sci.*, 1950, **32B**, 23–38.

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THE COMPARATIVE HISTOLOGY OF THE ALIMENTARY CANAL OF CERTAIN FRESH WATER TELEOST FISHES

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The work done on the histology of the alimentary canal of fishes of Europe and America has been summarised by Dawes (1929). Recently some valuable work has been done by Al-Hussaini (1946, 1947, 1949 *a*, 1949 *b*) on the histology, cytology and physiology of the gut of Teleost fishes from the Red Sea. Very little work has, however, been done on the histology of the alimentary canal of fishes of this subcontinent, the only noticeable contributions to the subject being those of Rahimullah (1935, 1936, 1939, 1941, 1943 and 1945), Dharmarajan (1936), Vanajakshi (1938), Sarbahi (1940) and Mohsin (1942, 1944–46).

The object of the present study is to find any possible correlation between the histological structure of the gut and the food and feeding habits of fishes. No work has so far been done with this object on the indigenous fishes. A study of the skin of the fishes has also been made as such a study greatly helps in the correct interpretation of the various structures in the lining of the buccal cavity and the pharynx. This factor has been completely neglected by previous workers on the histology of the alimentary canal and no use has been made of the extensive literature on the integument of fishes.

Material and Method

Living specimens of *Rita rita* Hamilton, *Cirrhina mrigala* Hamilton and *Ophicephalus gachua* Hamilton were obtained from fishermen. Small pieces of the various regions of the alimentary canal were fixed in Bouin's fluid or in Zenker-Formol for 16 to 18 hours. Pieces of skin from the head region were either fixed in Jenkin's decalcifying and dehydrating fixative or in Bouin's fluid for 16 to 20 hours. Satisfactory results were obtained with all these fixatives, though Bouin's fluid was preferred on account of its being easy to make, store, use and wash out.

The portions of the alimentary canal studied were from the buccal cavity, pharynx, œsophagus, stomach and intestine along with the rectum.

Tongue and pyloric cæca present in *Ophicephalus gachua* were also studied. Contents of the guts of all the available specimens of each fish were examined to verify the previous reports on the food of these fishes.

Food

Study of the contents of the stomach and intestine of *Rita rita* showed that two fishes had taken shrimp-like crustacea of fairly big size measuring over 1¼ inches in length, one had taken some insect larva, while the stomachs and intestines of two fishes were quite empty. *Rita rita* is carnivorous and as stated by Hamid Khan (1934) "it feeds mostly on insects, their larvæ and young fishes".

Ophicephalus gachua too is carnivorous and "destroys eggs, and fry of other fishes" (Hamid Khan, 1934). Mookerjee, Ganguly and Islam (1946) find that in the case of *Ophicephalus punctatus*, the fish when very young takes only unicellular and multicellular algæ. Later, they take to carnivorous diet consisting of Protozoa, Insecta, Copepoda, etc. As growth proceeds, the inclination towards fish-diet becomes evident, till in the adult, the percentage of fish in the diet of this fish may be upto 100%. *Ophicephalus gachua* is said to have a food similar to that of *O. punctatus*.

Cirrhina mrigala is a vegetarian fish but "has no objection to taking a bait of worms" (Hamid Khan, 1934). Mookerjee and Das (1945) describe *Cirrhina mrigala* as a bottom feeder taking lots of mud and sand. Mookerjee and Ghosh (1945) find that although adult *Cirrhina mrigala* "takes a mixed diet but the animal diet is almost negligible in comparison with vegetable..... . The major portion of the diet is the higher plant bodies in semi rotten condition".

Skin

The skin consists of epidermis and corium. A basement membrane usually separates the two regions.

Epidermis.—The epidermis of *Rita rita* is stratified and as described by Bhatti (1938) consists of ordinary epidermal cells, club-cells, mucus-cells, sensory cells and fibroblasts. The ordinary epidermal or epithelial cells (Fig. 1, *epc.*) are oval in shape with a large vesicular nucleus. In the superficial layers they tend to get flattened and lie parallel to the surface. The basal layer (*epb.*) rests on the basement membrane (*b.m.*) and consists of columnar squarish or rectangular cells. *Club-cells* (*c.c.*) are large oval or round cells having a central nucleus and cytoplasm which stains pink with eosin. In young cells the cytoplasm is homogeneous, but in older cells

small vacuoles may appear inside the cells or near the periphery. The club-cells vary considerably in size, form and arrangement in the skin from different regions of the body. Generally they are arranged in two or three rows. Some of the club-cells may be produced into a neck-like structure, but this neck never opens to the exterior. *Mucus-cells* or goblet cells (*m.c.*) are large round cells with basophilic reticular contents staining deeply with hæmatoxylin. Each mucus-cell has a crescentic nucleus lying at the bottom of the goblet-shaped body. The mucus-cells are scattered all over the epidermis, but are more common in the upper layers, where they may be seen opening to the exterior by a very short neck. *Sensory cells* generally occur in the form of end-buds, which are identical with the taste-buds found in the buccal cavity and pharynx. The taste-bud is an aggregation of sensory cells into a flask-shaped body situated on a finger-like projection of the corium. *Fibroblasts* are spindle-shaped cells with big granular nuclei lying in the basal layer of the epidermis.

In *Ophicephalus gachua*, the epidermis is thin and stratified. It consists of squarish or polygonal epithelial cells and numerous *sac-cells* (Fig. 2, *s.c.*). Club-cells are absent. The *sac-cells* are generally big cells, traversing more than three-fourth the thickness of the epidermal layer. Occasionally small sac-cells may be found in two or more layers. Very often the bigger cells can be seen to open to the outside by a short neck. These cells possess a very deeply-staining crescentic nucleus lying at the bottom of the sac. These cells differ from the typical mucus-cells in their staining reaction: the latter stain blue with hæmatoxylin, but the sac-cells fail to do so and pick up cytoplasmic stains. These sac-cells resemble closely the "*Sackformige serose Drüsen*" or "*sac-cells*" described by Bhatti (1938) in the integument of some Siluroidea. No sensory elements in the form of end buds seem to be present in the portion of the skin of *Ophicephalus gachua* studied. A distinct basement membrane is not present.

In *Cirrhina mrigala* the epidermis contains, in addition to the ordinary epidermal cells (Fig. 3, *epc*), numerous club-cells (*c.c.*) which have the typical structure exactly like those on the skin of *Rita rita.* At places the club-cells are seen protruding out of the general surface of the epidermis and may sometimes be seen completely ejected out. A similar condition has been described by Bhatti (1938) in some Siluroids and has been interpreted as indicating an excretory function. Mucus-cells, sensory elements and fibroblasts are not distinguishable in the region of the skin studied.

Corium.—In *Rita rita* the upper region of the corium (Fig. 1 *co.*) is frequently raised into papillæ projecting into the epidermis. The number,

height and diameter of these papillæ varies in the skin from different regions of the body. Lower down the fibres in the corium run parallel to the surface of the skin. In the lower region the lamellate character is better exhibted. A few bundles of vertical fibres may also be present in this region. Pigment cells and blood-vessels are often present below the basement membrane.

In *Ophicephalus gachua* the corium consists of highly vascular connective tissue and in it lie numerous scales which are also covered over by the epidermis (Fig. 2, *co.*).

The corium in *Cirrhina mrigala* consists of two well defined regions, an upper layer, just below the epidermis, consisting of very thick and closely packed connective tissue fibres that run parallel to the surface of the epidermis; and a lower layer of very loose and thin connective tissue fibres that run in all directions and enclose numerous fat cells. Blood vessels are present all over in this layer (Fig. 3, *co.*).

Buccal Cavity

The histological structure of the buccal cavity is essentially the same as that of the skin. The epidermis of the skin is represented here by the stratified *mucous epithelium* or *mucosa* and the corium by the *submucous connective tissue.*

Mucosa.—In *Rita rita* the bulk of the *mucosa* is formed of the ordinary epidermal cells (Fig. 4, *epc.*). Interspersed among these cells are the club-cells (*c.c.*), the mucus-cells (*m.c.*) and the sensory elements (*t.b.*). The *ordinary epithelial cells* are large and cubical on the surface, while in the region between the layers of the mucus cells and the club cells they are oval and lie parallel to the surface. The basal layer consists of squarish or rectangular cells with large spherical nuclei and rests on a thin basement membrane. The *club-cells* are closely packed in two or three rows, but are relatively fewer in number than in the epidermis of the skin. The *mucus-cells* are much commoner and form a more or less regular layer just below the surface. Some of the mucus-cells can be seen to open to the outside. Their contents stain light blue with hæmatoxylin. The *sensory cells* are in the form of globular or flask-shaped *end-buds* or *taste-buds*, situated on projections of submucosa. They consist of a bundle of elongated cells with their outer ends more deeply stained with eosin, and with an oval nucleus in each cell, and are similar in structure to *taste buds* described by Dawes (1929) and Rogick (1931) in fishes studied by them.

In *Ophicephalus gachua* the *mucosa* has numerous *sac-cells* (Fig. 5, *s.c.*) in addition to the oval ordinary epithelial cells (*epc.*). The basal layer rests

on a structureless basement membrane. The sensory elements take the form of flask-shaped taste-buds containing only a few sensory cells, and are very few in number.

In *Cirrhina mrigala* the mucus-cells are present in very large number in the superficial region of the *mucosa*. They may be arranged in two or more rows, but large areas may be completely devoid of them. The *taste-buds* and *club-cells*, though typical in structure are extremely rare. In size the club-cells do not reach the size of those found in *Rita rita*. *Fibroblasts* are numerous, and are found in the basal layer which rests on the basement membrane.

Submucosa.—In *Rita rita* the submucosa of the buccal cavity is a very thick coat, the upper region of which consists of compactly arranged connective tissue fibres running parallel to the surface immediately below the basement membrane and forming *stratum compactum* (Fig. 4, *str. comp.*), and with frequent vertical bundles. Lower down the submucosa becomes loose. Blood vessels are frequently seen all over the layer. A muscular coat, as a distinct part of the buccal lining, is absent.

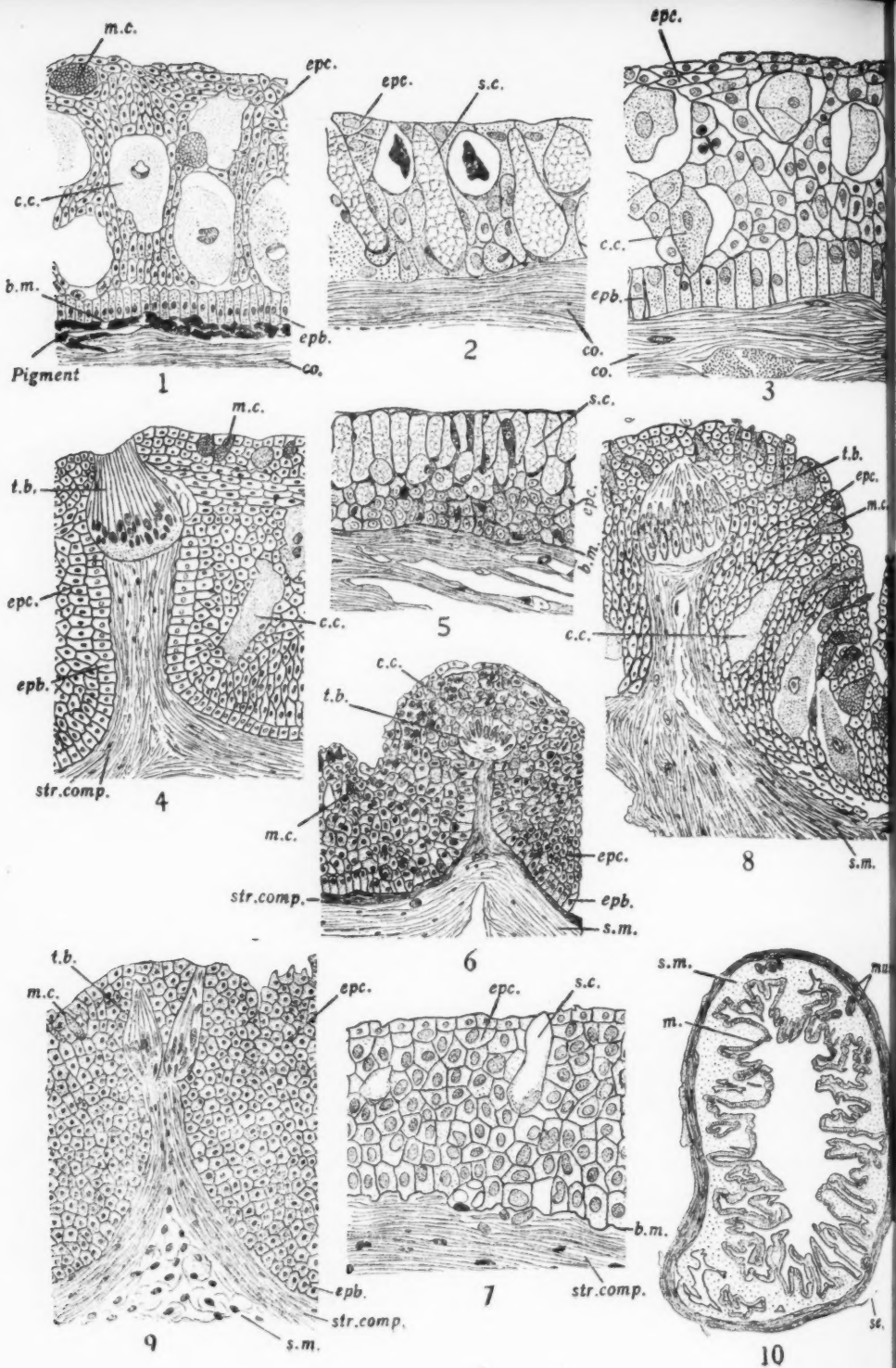
In *Ophicephalus gachua* two layers can be distinguished in the *submucosa*: an upper consisting of loosely arranged thin connective tissue fibres and a lower consisting of thick fibres. A stratum compactum is not present. Some of the fibres may run down to separate muscle fibres from each other. The muscles of the buccal cavity are striated.

In *Cirrhina mrigala* immediately below the basement membrane is the *stratum compactum* (Fig. 6, *str. comp.*), consisting of a compact layer of thick connective tissue fibres. In the lower strata the fibres of the submucosa enclose fat cells. Blood vessels and nerves are also present.

Tongue.—Tongue is present in *Ophicephalus gachua*. It is an oval structure consisting of *mucosa* (Fig. 7) and *submucosa* forming almost complete coats round the central cartilaginous support. The histological structure of these coats is the same as that of the buccal cavity, with the difference that a basement membrane (*b.m.*) and a stratum compactum (*str. comp.*) are very clear and a few typical *taste-buds* are present. The *taste-buds* consist of flask-shaped structures formed by a few elongated cells and situated on slight elevations of stratum compactum. They open to the outside. The *submucosa* is richly supplied with blood vessels. Its middle region is full of fat cells.

Pharynx

In general the histological structure of the pharynx is the same as that of the skin and the buccal cavity, except that a well developed *muscularis* is present in addition to the *mucosa* and *submucosa*.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FIGS. 1–10.—Fig. 1. *Rita rita:* V.S. Skin (×257). Fig. 2. *Ophicephalus gachua:* V.S. Skin (×575). Fig. 3. *Cirrhina mrigala:* V.S. Skin (×575). Fig. 4. *Rita rita:* V.S. Buccal cavity (×55). Fig. 5. *Ophicephalus gachua:* V.S. Buccal cavity (× 265). Fig. 6. *Cirrhina mrigala:* V.S. Buccal cavity (×257). Fig. 7. *Ophicephalus gachua:* T.S. Tongue (×575). Fig. 8. *Rita rita:* T.S. Pharynx (×257). Fig. 9. *Cirrhina mrigala:* V.S. Pharynx (× 257). Fig. 10. *Rita rita:* T.S. Oesophagus (× 17).

Mucosa.—In *Rita rita* the *mucosa* of the pharynx contains, in addition to ordinary epithelial cells, a single layer of *club-cells*, which are generally elongated with a smooth outline. The *mucus-cells* are present in large numbers, giving in some regions the appearance of stratification. The mucus-cells are chiefly concentrated in the superficial layers, and some of them can be seen to open to the exterior. Their secretion is often present in the lumen of the pharynx. The *taste-buds* (Fig. 8, *t.b.*) are more frequent than in the buccal cavity.

In *Ophicephalus gachua* the *mucosa* of the pharynx has the same structure as that of the buccal cavity with the difference that the sac-cells are more numerous. The taste-buds, though not very common, are however frequent than in the buccal cavity. A distinct basement membrane is absent.

In *Cirrhina mrigala*, the *mucosa* of the pharynx (Fig. 9, *m.ep.*) consists, as usual, of ordinary epithelial cells; mucus-cells, which are very numerous; taste-buds, having typical structure; and numerous fibroblasts scattered all over the epidermis.

Submucosa.—In *Rita rita* the *submucosa* of the pharynx is well developed, compact and muscular. Scattered all over the submucosa are numerous muscle bundles, fewer in upper layers but more in deeper layers. The submucosa thus passes insensibly into the muscle layer or *muscularis*, which consists of bundles of circular and longitudinal, generally striated muscles, lying without any regular arrangement. The fact that the circular and the longitudinal muscles do not form regular tunics, has been mentioned by Curry (1939) for the pharynx of *Cyprinus carpio communis*.

In *Ophicephalus gachua* a distinct stratum compactum is absent. The *submucosa* contains numerous fat-cells, nerves, blood vessels and stray bundles of apparently smooth muscle fibres.

In *Cirrhina mrigala* stratum compactum is narrow, and immediately below the submucosa consists of loose connective tissue in which numerous striped muscle fibres lie in criss-cross fashion. In the lower region of the submucosa the muscle fibres lie in bundles, and in this region there are numerous fat cells. Nerves and blood vessels are present in both the layers. A regular muscle coat is absent.

Oesophagus

The œsophagus, stomach and intestine have their wall composed of 4 coats, known respectively from without inwards as: *Serosa*, *Muscularis*, *Submucosa* and *Mucosa* or *mucous membrane*. The first three coats are of very similar structure in all parts of the gut, while the *mucosa* has a characteristic structure in the different regions.

Serosa.—In *Rita rita* the *serosa* or the *serous coat* of the œsophagus (Fig. 10) is very thin and is almost indistinguishable at places. The great bulk of the serous coat is formed of the subserous connective tissue, the bundles of which even pass into the muscularis. The serous epithelium which usually consists of a single layer of cells (Dawes, 1929; Rogick, 1931, Curry, 1939), is not distinguishable in *Rita rita*. The serous coat gives the appearance of stratification, probably due to a resemblance between the nuclei of the serous epithelium and those of the subserous connective tissue. Many blood vessels supply the serosa.

In *Ophicephalus gachua* (Fig. 11) the serous coat is quite conspicuous and encloses nerves and blood vessels. In *Cirrhina mrigala* the serous coat is extremely thin. At places, the subserous connective tissue is well developed and encloses nerves and blood vessels.

Muscularis.—In *Rita rita* both the muscle coats, an outer muscularis longitudinalis and inner muscalaris circularis, are fairly well developed and seem to be composed of unstriped muscle fibres (Fig. 10, *mus.*). Purser (1929) who describes a similar condition in *Calamoichthys calabaricus*, says that the extent of the striated muscle fibres is used by many authors for determining the boundary between the pharynx and the œsophagus. Blood vessels are sometimes present between the two layers or in the muscularis longitudinalis.

In the œsophagus *Ophicephalus gachua*, contrary to the condition in other parts of the alimentary canal, the muscularis circularis lies outside the muscularis longitudinalis. The fibres of the longitudinal muscles often wander into the outer region of the submucosa. Both striped and unstriped muscles are met with. The muscularis passes insensibly into the submucosa.

In *Cirrhina mrigala* the muscularis consists of circular muscle coat only (Fig. 12, *mus. c*). The muscularis longitudinalis is absent as a distinct outer coat. Numerous longitudinal muscle fibres, however, lie in the meshes of the submucosa, a condition also reported by Curry (1939) in *Cyprinus carpio communis*, Mohsin (L 944–46) in *Anabas testudineus* and by Rogick (1931) in *Campostoma anomalum*. The muscularis circularis is well developed and consists of very fine striated muscle fibres lying in quite a loose

manner. Fibres of connective tissue are often present in this layer and enclose minute blood vessels.

Submucosa.—The submucosa in *Rita rita* (Fig. 10, *s.m.*) is extensive and vascular, and enters the various folds of the mucous membrane. Its outer portion is loose, but the portion adjacent to the mucosa is somewhat denser and more deeply stained. No muscle fibres are present in this coat. In *Ophicephalus gachua* the muscularis passes insensibly into the submucosa. The region of the submucosa adjacent to the mucosa is free of muscles and contains numerous blood vessels. In *Cirrhina mrigala* the submucosa (Fig. 12, *s.m.*) is poorly developed and consists of very loosely arranged connective tissue fibres, scattered among which lie numerous longitudinal muscle fibres (*mus. f.*).

Mucosa.—In *Rita rita* the mucosa or the mucous membrane (Fig. 10, *m.*) is deeply folded, the folds often simulating tubular glands. A muscularis mucosæ and a tunica propria are not present. A basement membrane is also not distinguishable. The mucous epithelium consists of long columnar cells with oval nuclei. Mucus-cells are round or sac-like and are present in large numbers and can sometimes be seen opening to the exterior.

In *Ophicephalus gachua*, too, the mucosa is thrown into very high folds, which exhibit a tendency to branch. Muscularis mucosæ is absent. The mucous epithelium (Fig. 11, *m.ep.*), though definitely simple at places especially at the top of the folds, appears to be stratified at most places. Almost the whole of the mucous membrane is formed of very closely packed sac-cells, (*s.c.*), which lie in one or more layers. At places unmodified epithelial cells lie as supporting cells below the sac-cells. The older sac-cells appear to be empty, but some typically goblet-shaped deeply-staining oxyphilic cells, probably younger sac-cells are also present.

In *Cirrhina mrigala* the mucosa is simple, and is composed of a single, but greatly folded layer of ordinary epithelial cells (Fig. 12, *epc.*), with goblet —or mucus-cells (*m.c.*) and occasional taste-buds (*t.b.*). Some of the folds may be simple, others are branched. The ordinary epithelial cells are restricted to the tops and sides of certain folds. At other places they have either been replaced or squeezed in between the mucus-cells, which form the commonest element in the œsophageal mucosa of *Cirrhina mrigala.* Typical taste-buds are found here and there, especially near the top of the folds, and their presence in the œsophageal mucosa of this fish is a remarkable phenomenon and is presumably corelated with the feeding habits of the fish.

Stomach

Essentially the structure of the stomach is quite similar to that of the œsophagus except for the modification of the lining epithelium of the stomach to form the characteristic gastric epithelium and the gastric glands. Of the three fishes studied, *Cirrhina mrigala* does not posses a true stomach. In the other two fishes, the wall of the stomach consists of *serosa*, *muscularis*, *submucosa* and *mucosa*.

Serosa.—In *Rita rita* the serous coat of the stomach is similar to that of the œsophagus, and often encloses blood vessels and nerves. In *Ophicephalus gachua*, the serosa or the serous coat is extremely thin (Fig. 15, *se*.) and at most places consists of serous epithelium only.

Muscularis.—Serosa is followed by *Muscularis*, which is composed of complete coats of longitudinal and circular muscles. The two coats are separated by an ill-defined layer of connective tissue enclosing blood vessels and a nerve-plexus called Auerbach's Plexus. The longitudinal coat of muscles is broken up into bundles separated by connective tissue. Fibres of connective tissue can also be seen running into the muscle layer. Muscularis longitudinalis is thin and in the region of the pyloric sphinctre it becomes so much reduced as to be almost indistinguishable, while the muscularis circularis increases greatly to form the sphincter.

In *Ophicephalus gachua*, the muscularis consists of almost equally thick muscularis longitudinalis (Fig. 15, *mus. l.*) and muscularis cirrcularis (*mus.c.*) composed entirely of unstriated muscle-fibres. The two coats are separated by a layer of connective tissue, whose fibres penetrate both the coats and also enclose nerves.

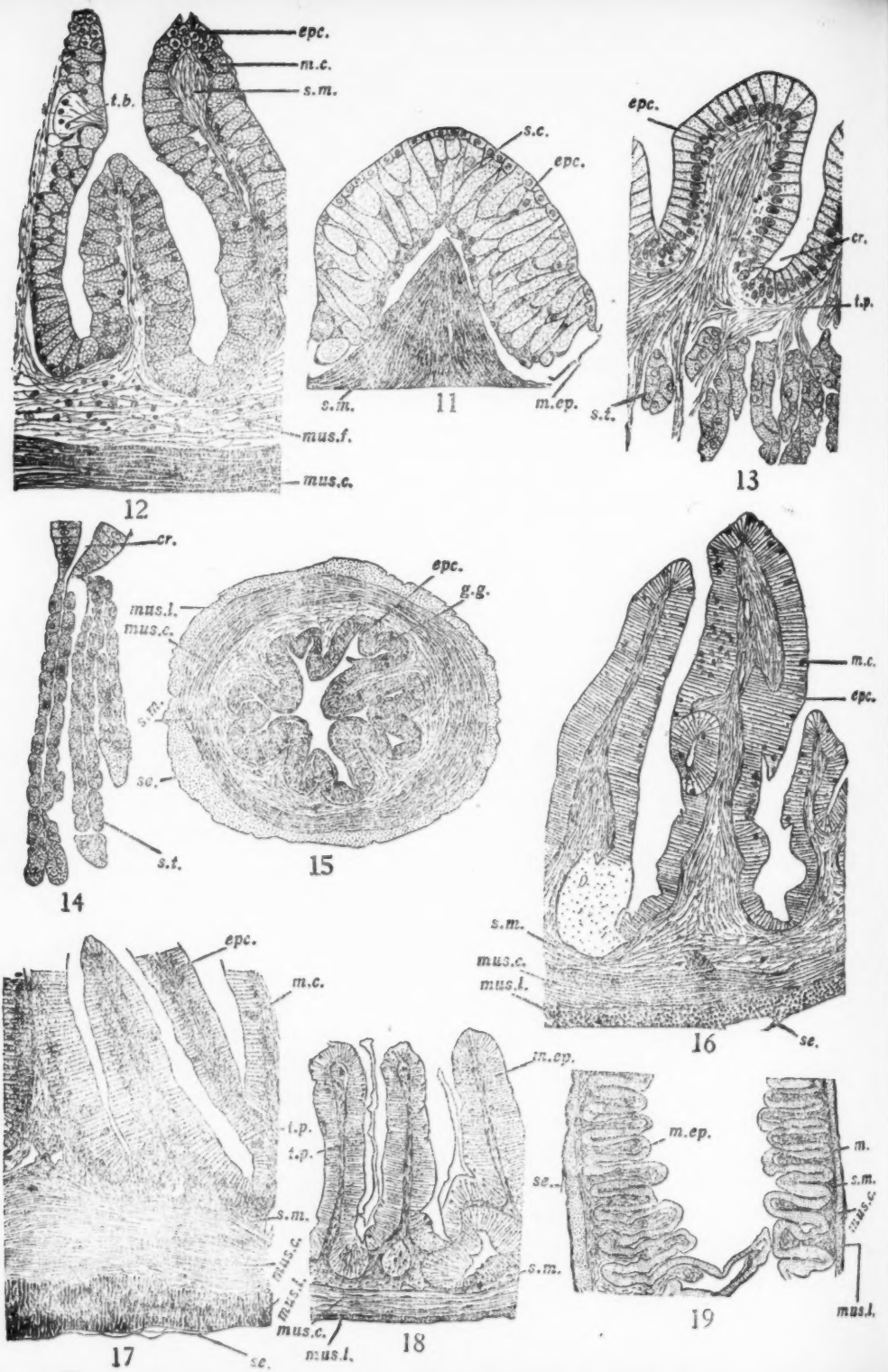
Submucosa.—In *Rita rita* numerous bundles of longitudinal muscle fibres lie scattered in the submucosa, especially in the region adjacent to the mucosa. But these muscles do not form a muscularis mucosæ. The submucosa is rich in blood vessels. The submucosa in *Ophicephalus gachua* is very well developed (Fig. 15, *s.m.*) richly vascular and invaded by numerous muscle fibres. These muscle fibres, mostly longitudinal, however, do not form a definite layer. The connective tissue fibres just below the submucous layer are said to comprise the *tunica propria* because they often penetrate into the mucous layer.

Mucosa.—The mucous membrane in *Rita rita*, consists of the lining epitheluim (Fig. 13, *epc.*), the gastric glands and the tunica propria. Stratum compactum is not present. The *tunica propria* is a vascular and well-defined layer of connective tissue, similar in structure to the submucosa. It

penetrates into the mucous layer, separating the gastric glands from each other, and supporting the lining epithelium. The *epithelium* consists of typical columnar cells frequently turning inwards to form the crypts of gastric glands. The epithelial lining consists of very long and narrow cells. As the epithelium turns inwards to form the crypt, the cells become short and broad, till at the bottom of the crypt they assume a cubical or cuboid form. The outer extremity of the epithelial cells stains deep blue with Mallory's stain. With mucicarmine, which is a specific mucous stain, this zone stains red. The cytoplasm of these cells appears more or less non-granular when stained with eosin or picro-indigo-carmine, but Mallory's stain brings out fine granulations. This granulation does not increase in any perceptible degree in the crypt cells, but the secretory cells are heavily granulated. In the region of pyloric sphinctre, where the gastric glands are absent, the free ends of the epitheliar cells stain deep red with mucicarmine, thus exhibiting a mucoid nature. The folds of the epithelium in this region may be taken to represent the crypts of the glands, with the secretory portions missing.

The *gastric glands* are of the simple tubular type, each gland (Fig. 14) consisting of one or more secretory portions or tubules (*s.t.*), opening into the bottom of the crypt (*cr.*). The lumen of the secretory tubule is very narrow and may even appear to be obliterated. Secretory cells of only one type are present, and have been described as the chief or the peptic cells by Greene (1912) and Blake (1930 and 1936) in the fishes studied by them. The absence of any differentiation into peptic (chief) and oxyntic (parietal) cells in fishes, was made a generalization by Edinger in 1877, (Blake, 1936). The secretory cells are large, round, oval or pear shaped with a very big and round nucleus. The cytoplasm is choked with granules, called zymogen granules, which are particularly well stained by Mallory's stain.

The *Mucous membrane* or *mucosa* in *Ophicephalus gachua* consists of gastric epithelial cells (Fig. 15, *epc.*), lining the lumen of the stomach, and gastric glands (*g.g.*), several of which lie in the region between the tunica propria and the gastric epithelium. The *gastric epithelium* consists of elongated columnar cells, narrow proximally, broad distally, and having most of the stainable protoplasm accumulated at the free, distal end. The protoplasm is faintly granular. The *gastric glands* consist of secretory portions or funduses and ducts of crypts. Many secretory tubules open to the exterior by one crypt. At the base of some crypts the cells remain unstained with Mallory's stain and pick up a very light stain with eosin.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FIGS. 11–19.—Fig. 11. *Ophicephalus gachua:* T.S. Oesophagus (×257). Fig. 12. *Cirrhina mrigala:* T.S. Oesophagus (×257). Fig. 13. *Rita rita:* T.S. Stomach (× 257). Fig. 14. *Rita rita:* A Gastric Gland (× 265). Fig. 15. *Ophicephalus gachua:* T.S. Stomach (× 17). Fig. 16. *Rita rita:* T.S. Intestine (× 55). Fig. 17. *Ophicephalus gachua:* T.S. Intestine (×55). Fig. 18. *Ophicephalus gachua:* T.S. Pyloric caecum (×17). Fig. 19. *Cirrhina mrigala:* L.S. Intestine (proximal part) (×29).

Similar cells in *Pleuronectes platessa* have been called "undoubtedly mucus producing cells, which can be compared to the mucoid cells scattered among the so-called peptic cells of the mammalian fundic glands" (Dawes, 1929). The cytoplasm of the secretory cells is full of zymogen granules, which stain red with Mallory's stain.

INTESTINE

The histological structure of the intestine is simple and resembles that of the œsophagus. The wall of the intestine consists of *serosa*, *muscularis submucosa*, and *mucosa*.

Serosa.—In *Rita rita* the serous coat of the intestine is vascular, (Fig. 16, *se.*). In *Ophicephalus gachua*, the *serosa* is very thin, being distinguishable only at places where it gets separated from muscularis to enclose blood vessels (Fig. 17, *se.*). In *Cirrhina mrigala*, the serous coat is extremely thin, without any subserous connective tissue and probably incomplete, (Fig. 19 and 20, *se.*).

Muscularis.—In *Rita rita*, both the muscle coats are present. The muscularis circularis (Fig. 16 *mus.c.*) is only slightly thicker than the muscularis longitudinalis (*mus. l.*). All the muscles are unstriped. The thin layer of loose connective tissue separating the two muscle coats encloses blood vessels and the nerve plexus of Auerbach. In *Ophicephalus gachua*, both the coats of muscularis are well developed, though the muscularis circularis (Fig. 17, *mus.c.*) is about four times as thick as muscularis longitudinalis *mus. l.*). Numerous blood vessels and nerves lie between the two coats, though there is no definite layer of connective tissue between them. In *Cirrhina mrigala*, muscularis circularis is generally four to five times thicker than the muscularis longitudinalis (Figs. 19 and 20, *mus.c.* and *mus. l.*). All the muscles are unstriped. The two coats are separated from each other by a zone which is traversed by protoplasmic threads and contains numerous blood vessels and nerves. These threads do not stain blue with Mallory's connective tissue stain.

Submucosa.—In *Rita rita* the submucosa (Fig. 16, *s.m.*) is not very well developed and consists of loose connective tissue containing numerous blood vessels. It is produced into finger-like processes covered over by the

lining epithelium to form the so-called "villi" True "villi" do not exist in any fish as is shown by the absence of the lacteals and the granular cells such as those of Paneth" (Rahimullah, 1945). The submucosa has also been designated as the "tunica propria", "combined parts of a true tunica propria and a submucosa" or merely as "areolar tissue" by various authors in the fishes described by them. In *Ophicephalus gachua*, the submucosa is loose, fairly well developed and richly supplied with blood vessels. In *Cirrhina mrigala*, too, submucosa is well developed and highly vscular (Figs. 19 and 20, *s.m.*).

Mucosa.—In *Rita rita*, the mucous membrane or mucosa consists only of a single layer of columnar epithelial cells (Fig. 16, *epc.*), interspersed amongst which are the mucus-cells (*m.c.*). A muscularis mucosæ, a tunica propria, a stratum granulosum and a stratum compactum are not distinguishable. The cells of the intestinal epithelium are of the simple columnar type, many times longer than broad, with slightly granular cytoplasm, and each with a well-defined top-plate. The nucleus lies towards the basal or attached end of the cell. The *mucus-cells* are present throughout the epithelium but their distribution is not uniform. They are round, oval or pear shaped with a filamentous basal portion which stains deeply with eosin and contains the nucleus. This indicates the evolution of the mucus-cells from the ordinary epithelial cells. The mucus-cell itself stains blue with hæmatoxylin or Mallory's stain.

In *Ophicephalus gachua* the mucosa is simple, consisting of a single layer of columnar epithlial cells (Fig. 17, *epc.*), resting on a well defined tunica propria (*t.p.*). No muscularis mucosæ, stratum compactum or stratum granulosum are present. The mucous membrane is greatly folded to form "villi", the core of which is formed by the submucosa as in *Rita rita*. The columnar epithelial cells are very narrow, have a deeply staining cytoplasm, and are provided with top-plates. Interspersed among the columnar epithelial cells are numerous mucus-cells.

In *Cirrhina mrigala*, the mucosa is thrown into folds, and the mucosal folds in the proximal region of the intestine proper differ from those in the greater length of the intestine by being transverse rather than longitudinal. The epithelium of the mucosa is simple consisting of ordinary epithelial cells (Fig. 21, *epc.*), in addition to the mucus-cells (*m.c.*). The ordinary epithelial cells are many times longer than broad, more markedly so in the distal parts of the intestine proper. Their nuclei are, consequently, compressed and oval. Top plates are present, though they are not so prominent in the distal part of the intestine proper. The mucus-cells are fairly

numerous and may be goblet-shaped, sac-shaped or tube-like, sometimes extending through the whole thickenss of the epithelium. The cells of the latter type indicate that the mucus-cells are formed by the transformation of the ordinary coulmnar epithelial cells. Numerous leucocytes are also strewn across the epithelium.

Pyloric Cæcum

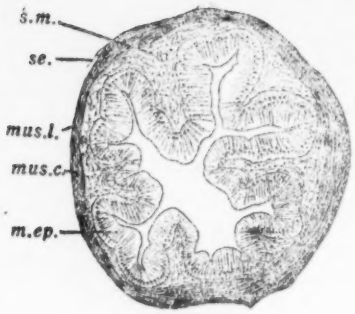
Two pyloric cæca are present in *Ophicephalus gachua* and their histological structure is very similar to that of the intestine (duodenum) of which they are the outgrowths (Fig. 18). Their *mucosa* consists of a layer of epithelial cells (*m.ep.*), resting on well-defined tunica propria (*t.p.*). The tunica propria consists of a compact layer of connective tissue. Rahimullah (1945) regards this layer as differentiated part of the submucosa, but Greene (1912) and others consider it a part of the mucous membrane and treat it accordingly. The mucosa is raised into finger-like folds, the core of which is formed by the submucosa. These folds have been termed as the "cæcal villi" by Rahimullah (1943) in contrast with the true villi. The cæcal villi are simple, unbranched and very long. The cæcal epithelium is simple and exactly like the intestinal epithelium. The stratified condition of the cæcal epithelium described by Rahimullah (1943) for *Ophicephalus striatus* and other species of this genus was, however, not observed.

Intestinal Bulb

In *Cirrhina mrigala*, the œsophagus passes into a long tube, slightly wider than the œsophagus and termed "the intestinal bulb" by Rogick (1931), or "the large arm of the intestine" by Curry (1939). The intestinal bulb gradually narrows down to the intestine proper, which forms by far the greater length of the gut.

Essentially, the histological structure of the intestinal bulb is similar to that of the rest of the intestine, the chief difference being in the degree of folding of mucosa. The *serosa* of the intestinal bulb (Figs. 22 and 23, *se.*) is very thin. In the *muscularis*, the circular (*mus.c.*) and the longitudinal (*musc. l.*) coats, though well developed, form only an insignificant part of the wall. The two coats, formed chiefly of unstriped muscle fibres, are separated by a thick pad of connective tissue containing nerves and blood vessels and a few striped muscles. A similar observation has been made by Curry (1939) in *Cyprinus carpio communis*.

The *submucosa* (*s.m.*) of the intestinal bulb is a very thin layer of coarse fibrous connective tissue, the processes of which pass into the mucosal folds



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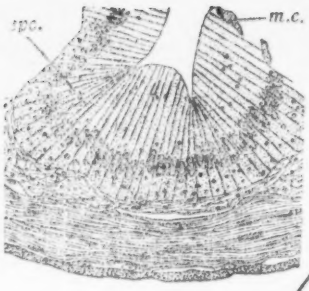

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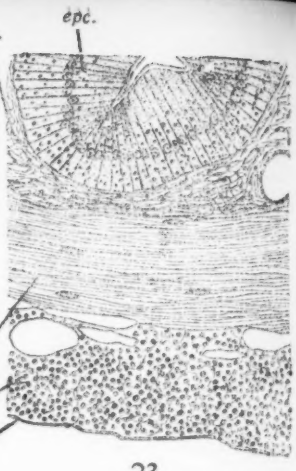

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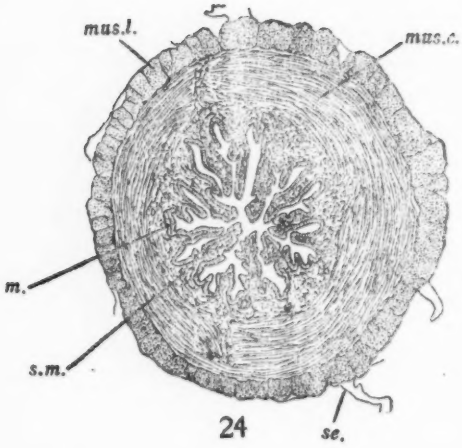

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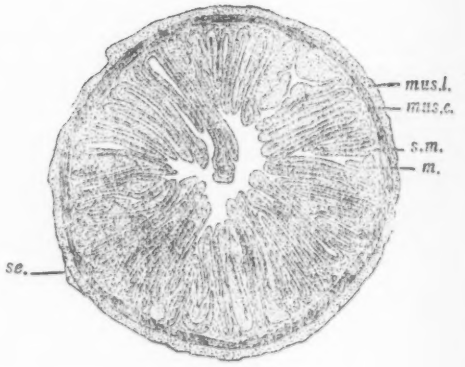

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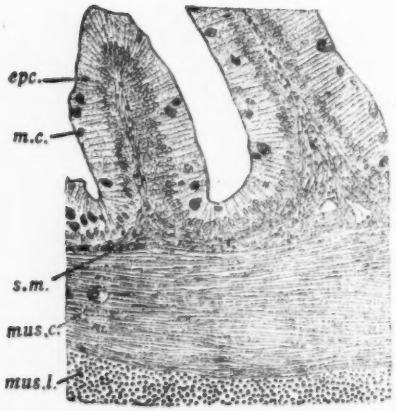

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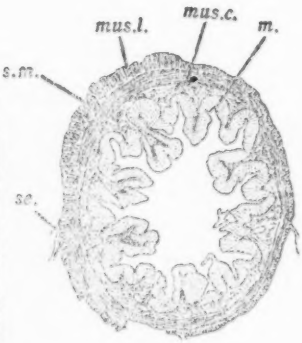

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FIGS. 20–27.—Fig. 20. *Cirrhina mrigala:* T.S. Intestine (middle and last part) (×55). Fig. 21. *Cirrhina mrigala:* T.S. Intestine (middle and last part) (×265). Fig. 22. *Cirrhina mrigala:* Intestinal Bulb (×11). Fig. 23. *Cirrhina mrigala:* T.S. Intestinal Bulb (257). Fig. 24. *Rita rita:* T.S. Rectum (×17). Fig. 25. *Ophicephalus gachua:* T.S. Rectum (×257). Fig. 26. *Cirrhina mrigala:* T.S. Rectum (× 29). Fig. 27. *Cirrhina mrigala:* T.S. Rectum (×575).

and carry numerous blood vessels. The *mucosa* shows an extensive degree of folding, the folds or villi being more than ten times as high as the three outer coats taken together. Posteriorly, however, the folds gradually decrease in size. Only ordinary columnar epithelial cells (*epc.*) and mucus-cells are present. The ordinary epithelial cells are extremely narrow and have most of their granular protoplasm aggregated near the free end of the cell. Top plates are present. Mucus-cells, though quite common, are not profuse.

RECTUM

The histological structure of the rectum resembles that of the intestine so closely that many authors do not describe rectum as a region distinct from the rest of the intestine.

Muscularis.—In all the three fishes studied, both the coats in the *muscularis* of the rectum are better developed than the corresponding coats in the intestine. The muscularis circularis (Figs. 24 and 26, *mus.c.*) is about three times as thick as the muscularis longitudinalis (*musc. l.*). Blood vessels and small nerves are present between the two coats. A definite layer of connective tissue does not exist between the two coats in the rectum.

Submucosa.—Submucosa in the rectum in all the fishes studied is well developed, but as in the case of the intestine, this layer may be regarded as representing the tunica propria or tunica propria and submucosa combined.

Mucosa.—The epithelial cells are exactly like the corresponding cells in the intestine. The mucus-cells are much more numerous than in the intestine. In *Ophicephalus gachua*, the mucous membrane of the rectum differs from that of the intestine in the fact that the " rectal villi " show a slight tendency towards branching. In *Cirrhina mrigala*, just below the *mucosa*, the connective tissue is compact and stains more deeply and may be regarded as *tunica propria*. The mucus-cells in the mucosa (Fig. 27, *m.c.*) are narrow, long and finger like with a narrow neck, but sometimes their middle or basal region may be swollen. They stain dark blue with hæmatoxylin, but fail to stain with Mallory's stain.

DISCUSSION

Skin.—Different types of gland-cells are met with in the skin of the three species studied. In *Rita rita* there are mucus-cells and club-cells; in

Ophicephalus gachua there are the sac-cells and in *Cirrhina mrigala* there are the club-cells. So far as the club-cells are concerned, it was pointed out by Oxner (1905) and later by Bhatti (1938) that the presence of club-cells is indicative of genetic relationship because these cells are absent in all Physoclisti and are present in all Physostomi, except the Salmonidæ and Loricariidæ. The present study corroborates this generalization in a striking manner. Thus *Rita rita* and *Cirrhina mrigala* both belonging to the group physostomi possess club-cells and *Ophicephalus gachua* belonging to the group Physoclisti is devoid of them, though *Rita rita* and *Ophicephalus gachua* are both carnivorous whereas *Cirrhina mrigala* is herbivorous. This shows that the club-cells in the skin, and hence in the buccal cavity and pharynx, indicate genetic relationship rather than an adaptation to feeding habits.

In *Cirrhina mrigala* some club-cells can be seen protruding out of the epidermis or even bodily ejected out, indicating the possibility of an excretory function of these cells. Wright (1884) attributed this condition to "defects in superficial layers of epidermis and due to the action of hardening reagents". Later observers (Oxner, 1905; Bhatti, 1938, and others) have, however, established the excretory function of these cells. Support or protection for the soft epidermis, is also advanced as a possible function of these cells.

Mucus-cells do not appear to be present in the portion of the skin of *Ophicephalus gachua* studied, and the sac-cells are the only type of gland-cells present. These sac-cells are exactly like the mucus-cells except for the staining reaction of the contents. Bhatti (1938) in the course of his studies on the integument of Siluroidea found that the sac-cells formed a definite generic character as they were present or absent in all the species of a genus. Presumably these cells also indicate a genetic relationship and are not concerned with feeding habits.

Terminal buds (taste-buds) have been observed in the skin of *Rita rita*, whose skin is naked, and are apparently absent in *Cirrhina mrigala* and *Ophicephalus gachua*, which have an exoskeleton of scales. Importance of taste-buds in feeding was first pointed out Herrick (1902) who wrote that "it may be regarded as established that fishes which possess terminal buds in the outer skin taste by means of these organs and habitually find their food by their means". This was amply confirmed by Hamid Khan (1934) who described a totally blind but robust and healthy specimen of *Rita rita* from the Ravi, proving that in this fish taste, and not sight, played the major role in feeding. *Ophicephalus gachua* is piscivorous in the adult condition and obviously sight is much more important than the sense of taste in hunting. *Cirrhina mrigala* has been described by Mookerjee and

Das (1945) as a Bottom-feeder taking lot of sand and mud. This mode of feeding does not require the presence of taste-buds in the skin of this fish.

Alimentary Canal.—The buccal cavity being a part of the embryonic stomodæum, has essentially the same structure as the skin. In *Cirrhina mrigala* mucus-cells appear in the buccal lining in large numbers, but the club-cells record a decrease in number in this fish as also in *Rita rita*. Taste-buds are present in the buccal cavity of all the three fishes, but there are comparatively very few in *Ophicephalus gachua*. This may easily be correlated with the nature of the food and the feeding habits.

A tongue is present in *Ophicephalus gachua*. As pointed out by Owen (1866) and Wiedersheim (1907), the tongue in fishes serves only as a tactile organ. The presence of a tactile organ in the buccal cavity of *Ophicephalus gachua* may be interpreted as a compensation for the absence of barbels and other integumentary sense-organs.

The pharynx, being a continuation of the buccal cavity, has the same structure as the latter. However, the mucus-cells in *Rita rita* and *Cirrhina mrigala*, and the sac-cells in *Ophicephalus gachua*, as well as the taste-buds in all the three fishes, show a marked increase in number in this region. The mucous membrane is thrown into low broad folds, probably in order to lodge a great number of gland-cells and taste-buds. An increase in gland-cells has been reported in many fishes, *e.g.*, *Cyprinus carpio* (Curry, 1939), *Campostoma anomalum* (Rogick, 1931). Probably the secretion of these gland-cells serves to wash the food down the œsophagus.

The œsophagus has the same four coats as characterise the rest of the alimentary canal. In the œsophagus there is a transition from the stratified to simple epithelium. Moreover, the folding of the mucosa becomes very well marked, "the complexity of folding is charactristic of this coat" (Blake, 1930). The œsophagus is richly supplied with gland-cell so much so that at places the gland-cells completely replace the ordinary epithelial cells. No taste-buds are present in the œsophagus except in *Cirrhina mrigala*. Probably this backward extension of the taste-buds can be correlated with the mode of feeding and the nature of food of this fish.

In *Rita rita* and *Ophicephalus gachua* there is a typical cæcal stomach provided with gastric glands. A true stomach, however, is absent in *Cirrhina mrigala*. The absence of a true stomach in *Cirrhina mrigala* has been correlated with its food. Absence of a true stomach in the Cyprinidæ has also been recorded by Wiedersheim (1907). Jacobshagen (1937) pointed out that a true stomach is absent, among others, in " pure carnivorous fishes

of which the intestine is shorter than the total length of the animal", and in " herbivorous fishes with an intestinal length of 5–6 times the length of the fish". In *Rita rita*, *Ophicephalus gachua* and *Cirrhina mrigala* the length of the gut is, respectively, 0·6, 0·7 and 11·25 times the length of the body. According to Jacobshagen's generalisation, all the three fishes should be stomach-less, but only *Cirrhina mrigala* comes up to expectations. Something more than the mere ratio between the lengths of the gut and the whole body should be sought for to explain the absence of a true stomach in some fishes.

In *Cirrhina mrigala* the function of storage, performed in other fishes by the stomach, has been taken over by the intestinal bulb which lies immediately behind the œsophagus and simulates a stomach. The intestinal bulb has been mistaken for a stomach by many authors (Mookerjee and Ghosh, 1945). However, its histological structure which is exactly like that of intestine, the opening of the bile duct immediately behind the œsophagus, and the absence of gastric glands, prove definitely that this organ is a part of the intestine.

The gastric glands of *Rita rita* and *Ophicephalus gachua* have the structure usual in the fishes, *i.e.*, they consist of (*a*) a crypt formed by an inturning of the gastric epithelium, and (*b*) a fundus or the secretory portion. The cells of the gastric epithelium have their outer ends stained red with mucicarmine, thus confirming the view of Stirling (1884), Jordan (1937) and others that these cells are mucoid in nature. The secretory cells are all of one type, confirming the statement of Edinger (1877) that in fishes in general there is no differentiation into peptic and oxyntic cells. The only type of secretory cells present is comparable, according to Edinger (1877), neither to the central nor to the parietal cells of Mammalia. Recent authors (Greene, 1912; Blake, 1930 and 1936; and others) compare these cells with the chief or peptic (Central) cells in mammalian gastric glands. The question of the source of acid is still unsettled. According to Stirling (1884) the superficial cells, in addition to producing mucus, also produce acid. This problem, however, needs further investigation.

The correlation between the nature of food and the length of the intestine is clearly shown by the fishes studied. The intestine of the two carnivorous fishes studied is shorter than the total length of the body. *Cirrhina mrigala*, which lives on organic particles in mud and sand, has an intestine about 11 times as long as the length of the body, probably because such food requires a very large surface for its absorption. Similar conclusions have been arrived at Al-Hussaini (1947) for other herbivorous fishes.

In *Rita rita* and *Ophicephalus gachua*, the intestine has, throughout, the structure typical of piscine intestine. In *Cirrhina mrigala* however, the intestine is differentiated into various regions. The first part has a wide diameter. It is called the intestinal bulb and its mucosa is thrown into very long folds. A short portion immediately behind the intestinal bulb has transverse folds, whereas in the rest of the intestine and the rectum the mucosal folds are longitudinal as is the usual condition.

Since the structure of the epithelium in all the three fishes is the same in all the regions of the intestine, no adaptive value can be attached to the intestinal mucosa. Goblet-cells or mucus-cells are present in the intestine of all the three fishes. In *Ophicephalus gachua* the mucus-cells are not met with in any other region of the alimentary canal except in the intestine.

In the fishes studied, pyloric cæca are found only in *Ophicephalus gachua*. As pointed out by Rahimullah (1945) " histologically, in general, the pyloric cæca are very similar to the intestine (= duodenum) of which they are outgrowths". As regards their function, Rahimullah (1945) says, "it is not yet certain what exact correlation exists between diet and the significance of these cæca in fishes. I think pyloric cæca have arisen in those fishes where the abdominal space has to be economised owing to the influence of some environmental factors". What factors have lead *Ophicephalus gachua* to have a short straight intestine and then to have two pyloric cæca as compensation, it is difficult to guess.

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SUMMARY

1. The comparative histology of the alimentary canal of *Rita rita* Ham., *Ophicephalus gachua* Ham., and *Cirrhina mrigala* Ham., has been studied to correlate the histological structure with the nature of food.

2. In addition to the ordinary epithelial cells, the epidermis of the skin contains mucus-cells and club-cels in *Rita rita*; sac-cells in *Ophicephalus*

gachua; and only club-cells in *Cirrhina mrigala*. Taste-buds are present only in the skin of *Rita rita*. In *Cirrhina mrigala* the club-cells have been seen being extruded bodily from the epidermis, thus providing a strong evidence for the view that the club-cells have an excretory function.

3. The histological structure of the buccal cavity and the pharynx is the same as that of the integument. Taste-buds and mucus-cells are present in the buccal cavity and pharynx of *Cirrhina mrigala*. In *Ophicephalus gachua* a few taste-buds are present on the tongue but none in the buccal and pharyngeal epithelia.

4. In the œsophagus the epithelium is simple. No club-cells are present in *Rita rita* and *Cirrhina mrigala*, but in *Ophicephalus gachua* sac-cells are profusely abundant. *In Cirrhina mrigala* a few taste-buds extend even upto the œsophagus. This has been correlated with the food of this fish.

5. A true stomach is present in the two carnivorous fishes *Rita rita* and *Ophicephalus gachua*, but in the herbivorous fish *Cirrhina mrigala* it is absent as shown by (i) the absence of gastric glands, (ii) opening of bile-duct just behind the œsophagus, and (iii) the part following the œsophagus having the typical structure of intestine. The absence of a stomach in *Cirrhina mrigala* has been correlated with its food. The cells forming the gastric epithelium in *Rita rita* and *Ophicephalus gachua* are mucoid in nature. Oxyntic cells are absent.

6. In *Cirrhina mrigala* the intestine is exceedingly long and coiled but in the other two fishes it is short and straight. This difference has been correlated with the difference in food in these fishes. In *Cirrhina mrigala* the first part of the intestine is dilated and is called the 'intestinal bulb'. In the region just behind the intestinal bulb the mucous membrane is transversely folded. In the rest of the intestine, as also in the intestinal bulb, the mucosa is longitudinally folded. No multicellular glands are present in the epithelium of the intestine. The mucus-cells (goblet-cells) are present in the intestine of all the three fishes studied. The mucus-cells make their appearance for the first time in the intestine in *Ophicephalus gachua*.

7. Pyloric cæca, present only in *Ophicephalus gachua* amongst the fishes studied, have the same histological structure as the intestine.

8. The rectum differs from the intestine only in having thicker muscular coats and more numerous mucus-cells.

Literature Cited

Al-Hussaini, A. H. .. .. "The Anatomy and Histology of the Alimentary Tract of the bottom-feeder, *Mulloides auriflamma* (Forsk.)," *J. Morph. Philad.*, 1946, **78.**

—— .. .. .. "The feeding habits and morphology of the alimentary tract of some teleosts," *Pub. Mar. Biol. Stat.*, Ghardaqa (Red Sea), 1947, 5.

—— .. .. .. "Functional Morphology of the Alimentary Tract of some fish in relation to differences in their feeding habits: Anatomy and Histology," *Quart. Jour. Micr. Sci.*, 1949, **90**, pt. 2.

—— .. .. .. "Functional Morphology of the Alimentary Tract of some fish in relation to differences in their feeding habits: Cytology and Physiology," *Ibid.*, 1949, **90,** pt. 4.

Bhatti, H. K. .. .. "The Integument and Dermal Skeleton of Siluroidea," *Trans. Zool. Soc.*, 1938, **24,** pt. 1.

Blake, I. H. .. .. .. "Studies on the Comparative Histology of the Digestive Tube of certain Teleost Fishes, I. The Sea Bass (*Centropristes striatus*)," *J. Morph. Philad.*, 1930, **50.**

—— .. .. .. "Studies on the Comparative Histology of the Digestive Tube of certain Teleost Fishes, II. A bottom-feeding Fish, the Sea robin (*Prionotus carolinus*)," *Ibid.*, 1936, **60.**

Chan, V. M. .. "The Histology of the Alimentary Tract of the Deep-water Gurnard, *Peristedion longispatha* (Goode and Bean)," *Univ. Nebraska Stud.*, 1941, **41,** No. IL.

Curry, E. .. .. .. "The Histology of the Digestive Tube of the Carp (*Cyprinus carpio communis*)," *J. Morph. Philad.*, 1939, **65.**

Dawes, B. .. .. .. "The Histology of the Alimentary Tract of the Plaice (*Pleuronectes platossa*)," *Quart. Journ. Micr. Sci.*, London, 1929, **73.**

Dharmarajan, M. .. .. "The Anatomy and Histology of the Alimentary System of *Otolithus ruber*," *Proc. Ind. Sci. Congr.*, 1936, **23.**

Edinger, L. .. .. .. "Ueber die Schleimhaut des Fischdarmes nebst Bemerkungen zur Phylogenese der Drusen des Darmrohres," *Archiv. fur Mikrosk. Anat.*, 1877, **13.**

Greene, C. W. .. .. "Anatomy and Histology of the Alimentary Tract of the King Salmon," Washington, D.C., *Dept. Comm. Bull., U.S. Bur. Fish.*, 1912 **32.**

Hamid Khan, M. .. .. "Habits and Habitats of Food-Fishes of the Punjab," *Journ. Bombay Nat. Hist. Soc.*, 1934, **37** , No. 3.

—— .. .. .. "On the relative value of certain Larvivorous Fishes from the Punjab, with Notes on their Habits and Habitats," *Indian Journ. Veter. Sci.*, 1943, **13,** pt. iv.

Herrick, C. J. .. .. "The Organ and Sense of Taste in Fishes," *Bull. U.S. Fish. Comm.*, 1903.

Jacobshagen, E. .. .. "Mittel-und-Enddarm," in Bolk and others' *Handbuch der vergl. Anat. der Wirbeltiere*, 1937, **3,** Berlin and Wien.

Jordan, H. E. .. .. "A Text-Book of Histology, New York, 1937, 7th Ed.

Mohsin, S. M. .. .. "A preliminary on the Morphology and Histology of an Air-breathing Fish, *Anabas testudineus* (Bl.)," *Proc. Ind. Sci. Congr.*, 1941, **28.**

——— .. .. .. "The Morphology and Histology of the Alimentary Tract of *Anabas testudineus*," *Journ. Osm. Univ.* (*Science Faculty*), 1944–46, **12.**

——— .. .. .. "Histology of the Alimentary Tract of a Fresh-water Goby, *Glossogobius guiris* (Ham.)," *Proc. Ind. Sci. Congr.*, 1946, **33.**

Mookerjee, H. K. and Das, B. K. "Gut of carnivorous and herbivorous fishes in relation to their food at different stages of their life," *ibid.*, 1945, **32.**

Mookerjee, H. K. and Ghosh, S. N. "Food of major Carps," *Ibid.*, 1945, **32.**

Mookerjee, H. K., Ganguly, D. N. and Islam, M. "On the composition of food and their correlation with weight and length of the body in the development of *Ophicephalus punctatus* Bloch.," *Ibid.*, 1946, **33.**

Oxner, M. .. .. .. "Ueber die Kolbenzellen in der Epidermis der Fische, ihre Form, Verteilung, Entstehlung und Bedeutung," *Jena Zeitschr. Naturw.*, 1905, **40.**

Owen, R. .. .. .. *On the Anatomy of Vertebrates*, Vol. I, *Fishes and Reptiles*, London, 1866.

Purser, G. L. .. .. "*Calamoichthys calabaricus*, Part II. The Alimentary and Respiratory Systems," *Trans. Roy. Soc. Edin.*, 1929, **56.**

Rahimullah, M. .. .. "A contribution to the structure and probable functions of the Pyloric Cæca in the family Opicephalidae," *Anat. Anz.*, Jena, 1935, **80.**

——— .. .. .. "On the Pyloric Cæca in the Notopteridae" (Abstract), *Proc. Ind. Sci. Congr.*, Calcutta, 1935, **22.**

——— .. .. .. "The structure of the Pyloric Cæcea in the family Mastacembelidae," *Ibid.*, 1936, **23.**

——— .. .. .. "On the structure and functions of the so-called Pyloric Caeca in two genera of Fishes, *Lactarius* and *Osphromenus*" *Ibid.*, Calcutta, 1938, **25.**

——— .. .. .. "On the disposition of the so-called Pyloric Caeca in a Brotu. lid fish, *Sirembo imberbis* (Tem. and Sch.)," *Ibid.*, 1941, **28.**

——— .. .. .. "Contributions to our knowledge of the Pyloric Cæca of three families of Fresh-water Indian Fishes (Ophicephalidae, Notopteridae and Mastacembelidae) together with some remarks on their probable functions," *Proc. Ind. Acad. Sci.*, 1943, **18** B.

——— .. .. .. "A comparative study of the Morphology, Histology and probable Functions of the Pyloric Caeca in Indian Fishes, together with a Discussion on their Homology," *Ibid.*, 1945, **21** B.

Rogick, M. D. .. .. "Studies on the Comparative Histology of the Digestive Tube of certain Teleost Fishes. II. A Minnow *Campostoma anomalum*," *J. Morph. Philad.*, 1931, **52.**

Sarbahi, D. S. .. .. "Alimentary Canal of *Labeo rohita*," *Journ. Asiat. Soc. Bengal* (Science), 1940, **5**, 2.

Stirling, W. .. .. .. "On the Chemistry and Histology of the digestive organs of Fishes," 2*nd Annual Report of the Fishery Board for Scotland*, 1884 (Quoted by Dawes, 1929).

Vanajakshi, T. P. .. .. "Histology of the Digestive Tract of *Saccobranchus vittatus*," *Proc. Ind. Acad. Sci.*, 1938, 7 B, 2.

Wiedersheim, R. .. .. *Comparative Anatomy of Vertebrates*, adapted by W. N. Parker, 1907.

Wright, R. R. .. .. "On the Skin and Cutaneous Sense Organs of *Amiurus*," *Proc. Canad. Inst.*, n.s., 1884.

Key to Lettering

b.m., Basement membrane; *c.c.*, Club-cell; *co.*, Corium; *cr.*, Crypt; *epb.*, Basal layer of epithelium; *epc.*, Ordinary epithelial cell; *g.g.*, Gastric gland; *m.*, Mucous membrane; *m.c.*, Mucus-cell (goblet cell); *m.ep.*, Mucous epithelium; *mus.*, Muscularis; *mus.c.*, Muscularis circularis; *mus.f.*, Muscle-fibre; *mus.l.*, Muscularis longitudinalis; *s.c.*, Sac-cell; *se.*, Serosa; *s.m.*, Submucosa; *s.t.*,Secretory Tubule; *str.comp.*, Stratum compactum; *t.b.*, Taste-bud; *t.p.*, Tunica propria.

MOLASSES AND *MOHUA* FLOWERS AS SOURCES OF ENERGY FOR AZOTOBACTER

By J. A. Daji, M. K. Patel, F.A.Sc., and M. M. Kibe

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In addition to its use as a soil amendment in the reclamation of alkali soils, the use of molasses has been advocated recently for increasing soil fertility. As a fertiliser, molasses has been reported to have given large increases in crop yields. However, with the exception of potash, it contains almost negligible quantities of the other two fertilising elements. Its main fertilising value, therefore, lies in the large amount of sugars that it contains. Substances rich in sugars and other available carbohydrates have been known to lead to greater fixation of nitrogen when added to the soil. Dhar and Sheshacharyulu (1936) obtained remarkable increases in nitrogen fixation by the use of molasses. Dhar and Mukherjee (1936) have shown that heaps of molasses and soil when exposed to sunlight serve to augment the nitrogen content of the soil. Bhaskaran, *et al.* (1939) have shown that a lime-molasses compound when added to the soil stimulates fixation of nitrogen content to the extent of one per cent. on the weight of the manure applied. It was therefore, decided to study the effect of molasses on the capacity of azotobacter to fix nitrogen when cultivated in pure culture. *Mohua* flowers (*Bassia latifolia*) which is another source of sugars were also included in the study. Table I gives the relevant data for both the substances used in the present study.

TABLE I

Percentage Composition of Molasses and Mohua Flowers

	Molasses	Mohua flowers (air dried)
Moisture ..	33·02	19·58
Reducing Sugars ..	14·56	46·50
Non-reducing sugars ..	39·70	14·80
Carbon ..	28·49	..
Nitrogen ..	0·058	0·490
Ash ..	13·92	3·32
Phosphoric acid (P_2O_5) ..	0·16	0·20
Potash (K_2O) ..	4·76	0·49
Lime (CaO) ..	2·30	0·02

Two sets were set up in duplicate, one with molasses and the other with *mohua* flowers. Ashby solution was used as culture medium with the

exception that mannite was substituted by molasses in one set, and by mohua flowers in the other. The organism was the same (Isolate III) employed by Uppal, Patel and Daji (1939). Each material was tried at several levels both in the presence and absence of sterilised soil. The bottles were incubated at room temperature for twenty days after which total nitrogen was determined in each case. Necessary controls were run at the same time. The results reportsed are the average of closely agreeing duplicates in each case. As the results of analysis of culture solutions, both with and without soil, confirm the previous findings of Uppal, *et al.* (1939), namely, that azotobacter fixes greater quantities of nitrogen in the presence of soil than in its absence, the figures for the amount of nitrogen fixed in the presence of soil only are reported in Tables II and III.

It will be noticed that both molasses and *mohua* flowers serve the same purpose as mannite in supplying energy to the organism. The organism fixes greater and greater quantities of nitrogen with increasing quantities of both the substances. In the case of *mohua* flowers, however, when the amount of *mohua* flowers added exceeds 1 gm., there is a drop in the amount of nitrogen fixed. At still higher levels (*i.e.*, 2 gm. and above) the organism does not fix any atmospheric nitrogen at all. There is, on the contrary, a certain loss of nitrogen from the system. Bhaskaran, *et al.* (1939) working with soil cultures to which a lime-molasses compound was added as a source of

TABLE II

Effect of Molasses on the Nitrogen-Fixing Power of Azotobacter

1 gm. soil + modified Ashby containing	Amount of Molasses added	Amount of carbon in molasses	Amount of nitrogen in molasses	Total nitrogen in culture solution	Nitrogen fixed	Net nitrogen fixed	Net nitrogen fixed per gm. of molasses	Net nitrogen fixed per gm. of carbon	Ratio of carbon to nitrogen fixed
1	2	3	4	5	6=(5−4)	7=(6−N in soil)	8	9	10
	gm.	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	
Culture (III) ..	..	..	..	1·29	1·29	0·33	..	..	..
,, +molasses	0·15	0·042	0·09	2·63	2·54	1·58	10·53	3·76	26·6
,, ,,	0·38	0·105	0·22	5·11	4·89	3·93	10·34	37·4	26·7
,, ,,	0·77	0·216	0·44	8·10	7·66	6·70	8·70	31·0	32·2
,, ,,	1·15	0·315	0·67	10·00	9·33	8·37	7·27	26·5	37·6
,, ,,	1·54	0·431	0·89	10·83	9·94	8·98	5·83	20·8	47·9
,, ,,	3·08	0·862	1·79	20·55	18·76	17·80	5·77	20·6	48·4

TABLE III

Effect of Mohua *Flowers on the Nitrogen Fixing Power of Azotobacter*

1 gm. soil + modified Ashby solution containing	Amount of *mohua* flowers added	Amount of nitrogen in *mohua* flowers	Total nitrogen in culture solution	Nitrogen fixed	Net nitrogen fixed	Net nitrogen fixed per gm. of *mohua* flower
1	2	3	4	5=(4−3)	6=(5−N in soil)	7
	gm.	mgm.	mgm.	mgm.	mgm.	mgm.
Culture (III)	..	..	1·16	1·16	0·68	..
„ + *mohua* flower	0·10	0·68	2·34	1·66	1·18	11·80
„ „	0·25	1·70	4·34	2·64	2·16	8 64
„ „	0·50	3·40	6·46	3·06	2·58	5·16
„ „	0·75	5·10	8·67	3·57	3·09	4·12
„ „	1·00	6·80	11·81	5·01	4·53	4·53
„ „	2·00	13·60	14·38	0·78	0·30	0·15
„ „	5·00	34·00	34·30	0·30	−0·18	−0·04
„ „	10·00	68·00	58·66	−9·34	−9·82	−0·98
„ „	20·00	136·00	130·10	−5·90	−6·38	−0·32

energy, have reported the occurrence of a similar phenomenon. They observed that the addition of this compound led to an increase in the amount of nitrogen in the culture up to a period of 12 days, after which the nitrogen content fell down steadily, indicating thereby a loss of fixed nitrogen from the system.

When the amount of nitrogen fixed per gram of the materials is considered, it will be seen that the nitrogen-fixing power of the organism increases at first with both molasses and *mohua* flowers, and this increase is followed by a sharp decline. It is a well-known fact that the presence of available nitrogen in the medium depresses the nitrogen fixing power of azotobacter. Though the amount of nitrogen added in the form of molasses (Table II) is very little, *i.e.*, ranging from 0·09 to 1·79 mgm., yet it has an appreciable effect in depressing the nitrogen-fixing power of the organism. In the case of *mohua* flowers (Table III), where the nitrogen added, especially at higher levels, is very large, the organism ceases to fix any nitrogen at all. Waksman (1931) reports that one-half milligram of nitrogen in an available form is sufficient to inhibit completely the fixation process.

Molasses and *mohua* flowers contain large quantities of sugars. It is quite possible that the decomposition products of these sugars, which are

likely to be mainly of an acidic nature, may also inhibit the nitrogen-fixing power of the organism. The nature and concentration of energy source are known to affect the activities of the azotobacter.

The amount of nitrogen fixed per gram of carbon also falls, after a small initial rise, with the increasing quantities of the energy substance. This is well illustrated in the case of molasses (Table II). In this respect, it behaves in just the same way as mannite, *viz.*, the more dilute the concentration of the energy source, the greater is the nitrogen-fixing efficiency of the organism.

In another series of experiments, two sets of culture flasks were set up to which mannite and molasses were added on the basis of their carbon content. The sample of molasses used in this series was different from the one used in the previous series, and contained 26·22 per cent. carbon and 0·211 per cent. nitrogen. It was not possible to include a similar set with *mohua* flowers as it was found almost impossible to get a uniform sample of the *mohua* flowers for the purpose of carbon estimation. Both the substances were added to culture solutions at several levels, to which 1·0 gm. of sterilised soil was added. The results (Table IV) indicate that at very low

TABLE IV

Effect of Mannite and Molasses on the Nitrogen-Fixing Power of Azotobacter

with mannite					with molasses			
Amount of carbon added in the form of mannite or molasses	Total nitrogen in culutre solution	Nitrogen fixed	Nitrogen fixed per gram of mannite	Nitrogen fixed per gram of carbon	Total nitrogen in culture solution	Nitrogen fixed	Nitrogen fixed per gram of molasses	Nitrogen fixed per gram of carbon
mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
0·395	6·26	4·64	4·64	11·74	10·65	5·86	3·90	14·70
0·197	4·67	3·05	6·10	15·48	5·96	2·76	3·68	14·00
0·098	3·23	1·61	6·44	16·43	3·80	1·38	3·68	14·00
0·039	2·74	1·12	11·20	28·72	2·60	0·68	4·53	17·40
0·030	2·38	0·76	10·10	25·33	2·47	0·65	5·77	21·66
0·020	2·06	0·44	8·80	22·00	2·22	0·38	5·06	19·00
0·010	1·82	0·20	8·00	20·00	1·78	0·07	1·87	7·00

Nitrogen in culture solution + soil = 1·62 mgm.
1 gm. of mannite = 0·395 gm. carbon = 1·5 gm. molasses.
Carbon in molasses = 26·22 per cent.
Nitrogen in molasses = 0·211 per cent.

concentrations, the amount of nitrogen fixed increases with the amount of energy material supplied. After a certain level, however, represented by 0·03 to 0·039 gm. of carbon, the organism becomes less active with increasing quantities of the material supplied. When molasses is compared with mannite, it will be seen that it is less efficient than mannite at almost all levels.

Summary

Both molasses and *mohua* flowers serve as source of energy for azotobacter, but the amount of nitrogen fixed per gram of material or per gram of carbon supplied is much less than in the case of mannite. In this respect, *mohua* flowers which contain larger amount of nitrogen than molasses are a greater offender. Even at very low concentrations, molasses is less efficient than mannite.

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References

Bhaskaran, T. R., Pillai, S. C. and Subrahmanyan, V. — *J. Ind. Inst. Sci.*, 1939, **22 A**, 127, 139, 149, 237.

Dhar, N. R. and Mukherjee, S. K. — *J. Ind. Chem. Soc.*, 1936, **13**, 155.

——— and Sheshacharyulu, E. V. — *Proc. Nat. Acad. Sci., India*, 1936, **6**, 99.

Uppal, B. N., Patel, M. K. and Daji, J. A. — *Ind. J. Agri. Sci.*, 1931, **9**, 689.

Waksman, S. A. .. .. — *Principles of Soil Microbiology*, 1931, Bailliere Tindall & Cox, London.

ON THE PROBABLE ORIGIN OF THE UNILOCULAR OVARY OF THE COMPOSITÆ FROM THE STYLIDIACEÆ

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The Australasian family Stylidiaceæ (Engler, 1908) includes the following six genera: *Donatia*, *Phyllachne*, *Forstera*, *Oreostylidium*, *Levenhookia* and *Stylidium*. The largest genus is *Stylidium* including 103 species. During the course of my embryological studies on the Stylidiaceæ (Subramanyam, 1950 *a*, *b*; 1951 *a*) certain important features were noticed in the structure of the ovary, which seem to throw some light on the origin of the unilocular ovary of the Compositæ.

In *Donatia Novae-Zelandiae* the inferior ovary is typically bilocular (Fig. 1) with the septum extending from the base of the ovary to the top and enclosing a number of ovules borne on the axile placenta. In the different species of *Stylidium* we find a series of stages showing the gradual loss of the septum separating the two locules and this starts from the top of the ovary towards the base. The loss of the septum may be due to its gradual dissolution or incomplete development. In *Stylidium graminifolium* (Subramanyam, 1951 *a*) the inferior ovary is bilocular in the lower region but becomes unilocular at the apex (Figs. 16–20). This is because of the incomplete septum or perhaps its dissolution in the upper region of the ovary. Correspondingly, the placentation is axile in the lower region and free central above. A similar condition is met with in *S. rupestre* (Fig. 2), *S. laricifolium* (Fig. 3), *S. lineare* (Fig. 5), *S. glandulosum* (Fig. 6), *S. hirsutum* (Fig. 7) and *Oreostylidium subulatum* (Fig. 4). Starting from *S. rupestre* and passing on to *S. hirsutum* we can trace a series of stages showing the gradual loss or incomplete extension of the septum (Figs. 2–7). Thus in *S. rupestre* the septum is incomplete at the apex of the ovary (Fig. 2); and in *S. hirsutum* there is no septum in the upper half of the ovary (Fig. 7). The next condition is reached in *Levenhookia dubia* (Fig. 10), *Stylidium scandens* (Fig. 11), *S. verticillatum* (Fig. 12) and *S. schoenoides* (Fig. 13), where the ovary is

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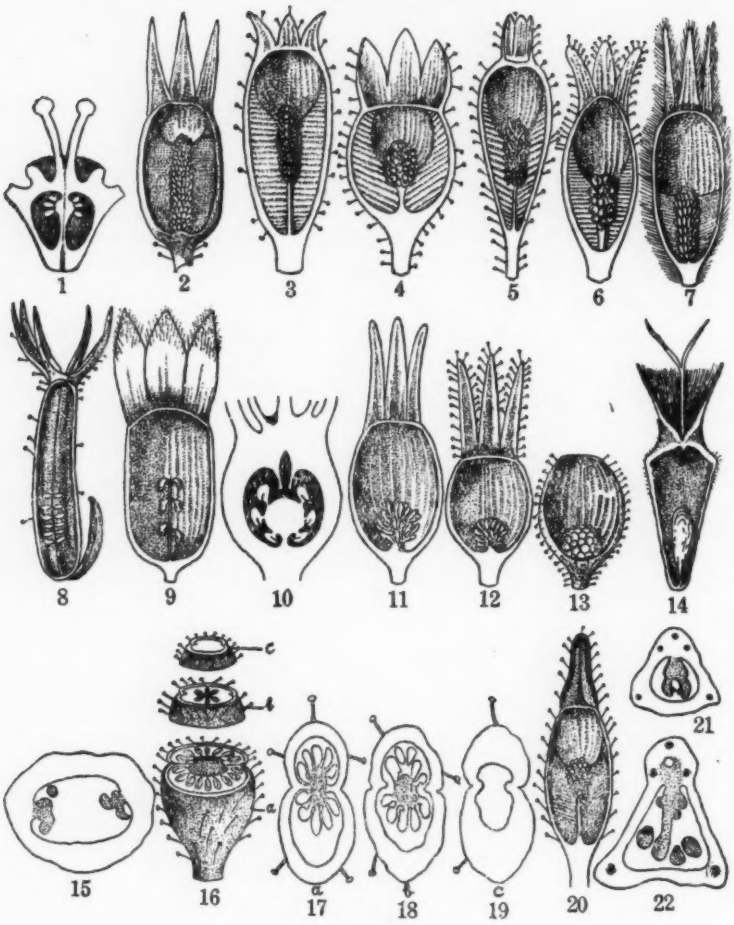

FIGS. 1–22

FIGS. 1–14. Longitudinal sections of ovaries of: *Donatia Novae-Zelandiae*; *Stylidium rupestre*; *S. laricifolium*; *Oreostylidium subulatum*; *Stylidium lineare*; *S. glandulosum*; *S. hirsutum*; *S. guttatum*; *S. imbricatum*; *Levenhookia dubia*; *Stylidium scandens*; *S. verticillatum*; *S. schoenoides*; *Zinnia*.

FIG. 15. T.S. of ovary of *Downingia*.

FIG. 16. A three-dimensional view of sections of ovary of *S. graminifolium* at different levels.

FIGS. 17–19. T.S. of ovary at levels *a*, *b* and *c* marked in Fig. 16.

FIG. 20. L.S. of ovary parallel to septum to show its unilocular nature at the top.

FIGS. 21–22. Transverse sections of two ovaries of *Stylidium adnatum* to show atrophied condition of the upper chamber.

[*Note*.—In Fig. 1 the longitudinal section is taken at right angles to septum; in Figs. 2–7 it is parallel to septum. Figs. 1, 2, 4, 6–9 and 11–13 after Engler (1908); Figs. 15, 21 and 22 after Rosén (1935); Figs. 3, 5, 10, 14, 16–20 original].

typically unilocular as a result of the complete loss of the septum. In these forms the ovules are borne on a free central placenta. Further, in *Levenhookia dubia*, *S. scandens* and *S. verticillatum* the short axis of the free central placenta projects into the locule from the base of the ovary (Figs. 10–12). In *S. schoenoides* even this is suppressed so that all the ovules are crowded at the base of the locule (Fig. 13). If at this stage there is a reduction in the number of ovules to one, the result will be a condition similar to that of the unilocular ovary of the Compositæ with a single anatropous ovule borne on a basal placenta (Fig. 14). In fact a tendency towards reduction in the number of ovules is also seen in certain members of Stylidiaceæ, *viz.*, *S. guttatum* (Fig. 8) and *S. imbricatum* (Fig. 9). Further, in these forms the axis of the free central placenta is very thin and slender.

There is also another possible line of approach towards the origin of the unilocular condition of the Compositæ. In *Stylidium graminifolium* (Subramanyam, 1951 *a*) where the ovary is bicarpellary, the anterior carpel with its locule is distinctly larger than the posterior (Figs. 16–19). Next, in *S. adnatum* Rosén (1935) finds that one of the chambers shows an atrophied condition and does not bear ovules (Figs. 21, 22). He considers it probable that the one-chambered ovary of the Stylidiaceæ has been derived from the typically two-chambered ovary of the Lobeliaceæ and adds that even among certain Lobeliaceæous members a unilocular condition of the ovary is met with as in *Downingia* (Fig. 15), though this condition is of a somewhat different nature from that of *Stylidium adnatum*. So, if in a form like *S. adnatum*, where one of the locules become atrophied, there is next a complete suppression of this locule, the result will be the unilocular condition of the Compositæ.

Conclusions

The unilocular ovary of the Compositæ from the Stylidiaceæ may have arisen by the gradual suppression or incomplete development of the septum separating the two locules or by abortion of one of the locules. On similar lines Rosén (1946) has derived the origin of the ovary in the Compositæ from the Goodeniaceæ. Hence the unilocular condition of the ovary in the Compositæ may be traced from two levels, *viz.*, the Stylidiaceæ and the Goodeniaceæ. It may be also pointed here that the families Lobeliaceæ, Stylidiaceæ, Goodeniaceæ and Compositæ are closely interrelated on embryological grounds (Rosén, 1935, 1937, 1946, 1949; and Subramanyam, 1950 *a*, *b*; 1951 *a*, *b*).

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Summary

It is suggested that the unilocular ovary of the Compositæ may have originated as a result of the gradual suppression or incomplete development of the septum separating the two locules in the ovary of the Stylidiaceæ. It is also possible that the unilocular condition of the Compositæ may have been derived as a result of abortion of one of the locules, for, this tendency is already evident in certain species of *Stylidium* like *S. adnatum*.

Literature Cited

Engler, A. .. *Das Pflanzenreich. Heft*, 1908, 35 B and IV, 278, 1–98.

Rosén, W. .. "Beitrag zur Embryologie der Stylidiaceen," *Bot. Notiser (Lund)*, 1935, 273–78.

———— .. "Beiträge zur Kenntnis der Embryologie der Goodeniaceen," *Acta Horti Götéborgs*, 1937, **12**, 1–10.

———— .. "Further notes on the embryology of the Goodeniaceæ," *Meddel. Fr. Göteborgs. Bot. Trägd.*, 1946, **16**, 235–49.

———— .. "Endosperm development in Campanulaceæ and closely related families," *Bot. Notiser (Lund).*, 1949, **2**, 137–47.

Subramanyam, K. .. "An embryological study of *Levenhookia dubia* Sond. in Lehm.," *Proc. Nat. Inst. Sci. India*, 1950 *a*, **16**, 245–53.

———— .. "Development of embryo-sac and endosperm in *Stylidium tenellum* Swartz.," *Curr. Sci.*, 1950 *b*, **19**, 294.

———— .. "A morphological study of *Stylidium graminifolium* Swartz.," *Lloydia*, 1951 *a* (in press).

———— .. "Flower structure and seed development in *Isotoma fluviatilis* F. vM.," *Proc. Nat. Inst. Sci. India*, 1951 *b* (in press).

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